Interleaved Recording of the Auditory Brainstem Response

A thesis submitted in partial fulfilment of the requirements for the Degree of

Master of Audiology

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Abstract

The auditory brainstem response (ABR) is a sound-evoked electrical potential produced by the auditory brainstem in response to transient acoustic stimuli. This response is measured clinically to obtain estimates of hearing sensitivity and identify neurological pathologies in the VIIIth nerve and ascending auditory pathway. At present, most recordings of the ABR that are performed in clinical settings utilise either one or two channels (usually corresponding to the right or left side of the head) and deliver acoustic stimuli sequentially. After a recording has been made using several thousand presentations of one type of stimulus, the clinician then commences testing using a different stimulus or intensity or delivers the stimuli to a different ear. The custom-written software our laboratory developed for evoked-potential recordings (O'Beirne, 2015) is capable of interleaving multiple types of stimuli to multiple transducers or destinations. This interleaving offers several theoretical advantages, which were investigated with 19 normal hearing participants. Comparisons were made between ABR waveforms obtained by i) interleaving high click-rate stimuli divided between both ears (e.g. 45.5/s to each ear, 90.9/s overall); ii) delivering stimuli monaurally at the slower single-ear rate (e.g. 45.5/s); and iii) delivering monaurally at the faster total rate (e.g. 90.9/s). Results showed that ABR waveforms obtained in the rapid interleaved condition were a) of comparable quality and amplitude to those from obtained in the monaural slow condition, despite being recorded in half the time; and b) were not affected by the significant increase in Wave V latency found with the faster monaural stimulation condition. Interleaving of stimuli between the ears allows for the reduction of overall ABR test time compared to the conventional sequential method of recording. This is potentially valuable in a clinical setting by enabling clinicians to complete a more thorough diagnostic testing within their allocated time.

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List of Abbreviations

ABR Auditory Brainstem Response

AEP Auditory Evoked Potential

ALR Auditory Late Response

AMLR Auditory Middle Late Response

AP Action Potential

ASSR Auditory Steady State Response

BIC Binaural Interaction Component

BM Basilar Membrane

CNS Central Nervous System

dB Decibels

Hz Hertz

IHC Inner Hair Cell

ISI Interstimulus Interval

MET Mechanoelectrical Transduction

MLS Maximum Length Sequence

MRI Magnetic Resonance Imaging

OHC Outer Hair Cell

pABR Parallel Auditory Brainstem Response

SNR Signal-to-Noise Ratio

Chapter 1: Introduction

1.1 General Introduction

The auditory brainstem response (ABR) is routinely used in clinical settings as an electrophysiologic method of determining auditory function. ABRs are a measure of transient-evoked electrical activity of the neurons in the vestibulocochlear (VIIIth cranial) nerve and auditory brainstem. The ABR waveform morphology, and more quantitatively the peak latency and amplitudes, are used as diagnostic indicators to estimate the hearing thresholds and presence of neurological pathologies (Starr, 1976; Stockard & Rossiter, 1977; Tsubokawa et al., 1980; Weber & Fujikawa, 1977). Clinically, obtaining these waveforms in a fast and efficient manner is often desired. This is important so that testing time may be saved, or a more thorough assessment may be performed. Clinicians are often allocated a limited time to complete the tests or are restricted by how long the newborn being tested is asleep during the ABR test (Burkard, Shi & Hecox, 1990). Failure to obtain a clear result within this limited time could potentially delay diagnosis or result in the patient being lost to follow-up.

Typically, most evoked potential software measures the ABR using one type of stimulus at a time (e.g. a click, chirp or a tone-burst of a particular frequency). Due to the small size of the signal (less than 1 μV), responses to thousands of stimuli must be averaged to increase its signal-to-noise ratio (SNR) (Bukard, Shi & Hecox, 1990). Doing so necessarily takes time. Furthermore, the components of the ABR waveform are affected by the parameters of the stimulus such as the intensity, frequency and rate of the stimuli being presented (Hall, 2007). There is a limit to how much the stimulation rate can be increased because the shape (or morphology) of the waveform deteriorates when these stimuli are presented too close together. This is due mainly to fatigue and adaptation of the peripheral

auditory nerve at very high activation rates (Don, Allen & Starr, 1977) – in particular, the specific neurons that are stimulated by that stimulus.

Interleaving stimuli that excite completely different populations of neurons could potentially permit the overall stimulation rate to be increased while avoiding overstimulating the same neurons. For example, a clinician may average 2000 responses to a 500 Hz tone-burst stimulus followed by 2000 responses to a 4 kHz tone-burst, both at 27 stimuli/s. Assuming these two tones stimulated different populations of neurons within the auditory pathway, we could instead present 4000 tone-bursts which alternate between 500 Hz and 4 kHz, and double the presentation rate to 54/s, and yet both recordings would be obtained in half the time. If the two populations of neurons are independent, the resulting waveform morphology could potentially be at the same quality as their sequential counterparts. Similarly, instead of two different tone-burst frequencies, we could stimulate two different ears using chirps or clicks, at twice the rate for example. Doing so in alternating fashion could half the recording time compared to sequential acquisition of waveforms from these two ears at the normal rate.

With this goal in mind, our laboratory has developed evoked potential software that is capable of fully interleaving these stimuli, both in terms of stimulus type and delivery ear (O'Beirne, 2015). Previous research that has investigated interleaved stimuli has generally interleaved different types of stimuli presented to the same ear whereas the present study will investigate the effects of interleaved click stimuli presented to both ears in a pseudo-simultaneous manner. Other studies that also evoke the ABR in both ears generally do not interleave the stimuli abut rather, present multiple stimuli simultaneously. For a given overall stimulus rate, the decreased stimulus rate experienced by each ear resulting from this interleaved method potentially offers the advantages of producing clearer waveform

morphology, which give clinicians additional diagnostic information than just the presence or absence of a response.

The aim of this study was to determine if the rapid interleaving of ABR stimuli with different characteristics and delivery modes offer practical advantages in terms of response quality (i.e. SNR and waveform morphology), test time, and diagnostic accuracy. ABR data was collected from 19 normal hearing adults. Due to time constraints, we focussed comparing ABR waveforms evoked by click stimuli delivered using an interleaved presentation paradigm to both ears with those evoked by monaural stimulation. Latency and amplitude measurements of wave V were analysed comparing monaural and interleaved binaural stimulation at different presentation rate combinations. Objective measurement of the waveform quality (the Fsp measure; Elberling & Don, 1984) was also obtained and compared for each stimulus condition. Descriptive statistics such as means and standard deviation for each measurement were calculated.

1.2 Anatomy and Physiology of the Hearing system

To understand how the proposed modifications to the ABR technique could affect the waveforms that are measured, it is important to review the anatomy and underlying physiology of the hearing system that generates the ABR.

The overall function of the hearing system is to allow the listener to detect and perceive sound waves in their environment. Human hearing permits the perception of speech and other acoustic information as well as the localization of the sound sources (Emanuel, Maroonroge & Letowski, 2009). The auditory system encompasses the peripheral auditory system and the central auditory nervous system.

1.2.1 The outer ear. The peripheral auditory system is divisible into three main parts: the outer, middle and inner ear. Starting with the most lateral division, the outer ear consists of

the pinna (or auricle) and the auditory canal (or external auditory meatus). The pinna is composed of skin covered cartilage and its ovoid shape and uneven surface allows it to collect, direct and modify (filter) sounds as they enter the ear canal. The topography of the pinna thereby aids with the localisation of sounds, as sounds of different frequencies are boosted or attenuated by reflections from its ridges (Batteau, 1967). The sound waves are then funnelled through the ear canal where they undergo further alterations due to the specific resonance characteristics of the ear canal (Emanuel, Maroonroge & Letowski, 2009; Shaw, 1974). The outer one-third of the ear canal is surrounded by cartilage whereas the medial two-thirds are surrounded by the temporal bone. Together, the two sections of the typical adult ear canal are approximately 25 mm in length (Alvord & Farmer, 1997). The skin of the ear canal is continuous with the tympanic membrane or ear drum, which vibrates in response to waves, converting acoustic energy into mechanical energy (Pickles, 2013; Maroonroge, Emanuel & Letowski, 2009).

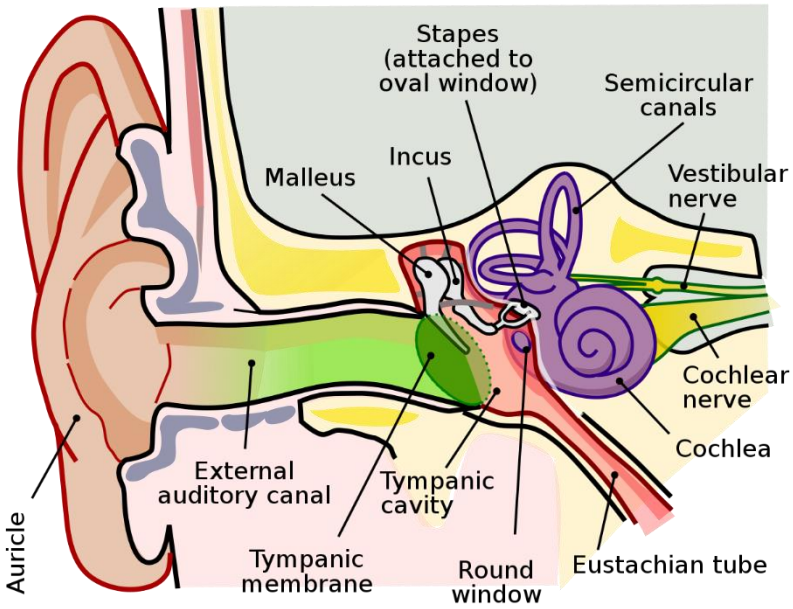

Figure 1. Diagram of the peripheral auditory system. Retrieved from "Perception Space- The Final Frontier", by L. Chittka and A. Brockmann, 2005, PLoS Biology. (https://doi.org/10.1371/journal.pbio.0030137). Copyright 2005 by Chittka and Brockmann.

1.2.2 The middle ear. The eardrum is a thin oval membrane with the shape of a shallow cone marking the border between the outer and middle ear (Alvord & Farmer, 1997). The ear drum consists of three layers, the lateral layer being an epidermal layer which has cells that migrates outwards from the centre of the ear drum to aid in clearing debris and wound healing. The intermediary layer is a fibrous layer containing type II and type III collagen and the medial layer is a mucosal epithelial layer continuous with the middle ear cavity (Lim, 1995). The surface of the ear drum is divided into two areas, the pars tensa and the pars flaccida (which lacks the fibrous intermediary layer). The vibration of the ear drum transfers this acoustic energy to the ossicles in the air-filled middle ear cavity. The three tiny bones constituting this ossicular chain are the malleus, incus and stapes and are the smallest bones in the body. The malleus is connected to the pars tensa portion of the ear drum and the stapes articulates with the oval window of the cochlea. In between these two bones is the incus (Pickles, 2013). The ossicles are held in place by ligaments and are attached to small muscles which contract in response to loud sounds. The stapedius muscle which is attached to the neck of the stapes and the tensor tympani which is attached to the malleus contract to temporarily stiffen and reduce the efficiency of the ossicles. The contraction of these muscles is termed the middle ear or acoustic reflex and is thought to provide limited protection to structures of the inner ear from exposure to intense sounds (Møller, 1962; Moore, 2003). The middle ear acts as an impedance-matching transformer to greatly improve the transfer of energy between the vibration of the ossicles in an air medium to the movement of inner ear fluids. This is achieved mainly by the difference in the area of the ear drum and the oval window, with the area of the ear drum being 17 times larger than the oval window. Other mechanisms for the efficient energy transfer are the lever action of the ossicles and buckling effect of the ear drum (Aibara, Welsh, Puria & Goode, 2001; Moore, 2003; Zwislocki, 1962).

1.2.3 The inner ear. The inner ear is located medial to the middle ear inside a bony cavity called the bony labyrinth. The three main parts of the inner ear are the semi-circular canals, the vestibule and the cochlea. The snail-like cochlea is the organ of hearing whereas the vestibule and the three semi-circular canals are the organs which contribute to the sense of balance (Ekdale, 2016). Within the bony labyrinth is a lining of membranous labyrinth of interconnected soft tissue and this arrangement forms three chambers in the cochlea: scala vestibuli, scala media and scala tympani. These chambers or ducts are filled with either endolymph (scala media) or perilymph (scala vestibuli and scala tympani). The cochlear endolymph is supplied by the stria vascularis and is high in potassium (157 mM) and low in sodium (Wangemann & Schacht, 1996). In contrast, the composition of perilymph is similar to extracellular fluid, which is rich in sodium (148 mM) but low in potassium (Echteler & Fay, 1994; Sterkers, Ferrary & Amiel, 1988; Wangemann & Schacht, 1996).

The mechanical energy from the vibration of the stapes is transferred into the movement of the inner ear fluids of the cochlea. The stapes is attached to the oval window at the base of the cochlea which leads into scala vestibuli. The movement of the stapes transfers sound energy into the cochlear fluids and causes the alternating movement of the round window which terminates the scala tympani at the base of the cochlea. In between scala tympani and scala vestibuli lies scala media, along which the basilar membrane (BM) runs. The longitudinal vibrations in the cochlear fluids give rise to a transverse wave along the BM (Moore, 2003). The wave propagates from the base of the BM to the apex, peaking at different areas depending on the spectral characteristics of the sound stimulus. The frequency response of different points along the BM is due to its varying mechanical properties from the base of the apex: The base of the BM is narrow and stiff, causing high frequency sounds to produce their maximal displacement in this region, while the wide and thick region at the apex of the BM is where low frequency sounds cause the greatest displacement (Von Bekesy

& Wever, 1960; Moore, 2003; Ren, 2002). This pattern of tonotopicity in the cochlea is maintained throughout the ascending auditory pathway (Mann & Kelley, 2011).

Perched on the BM is the organ of Corti, which converts the mechanical motion of the BM into electrical signals that travel through the auditory nerve. This process is termed mechanoelectrical transduction (MET), and is performed by the hair cells (Pickles & Corey, 1992). The hair cells in the organ of Corti are distributed longitudinally along the BM and consist of two types: inner hair cells (IHCs), of which there are approximately 3500, and the outer hair cells (OHCs), which number approximately 12,000 (Ekdale, 2016; Maroonroge, Emanuel & Letowski, 2009; Wright et al., 1987). The hair cells are named after the stereocilia which project from their apical surface. Following the transmission of sound through the outer and middle ear, transverse movement of the BM causes a relative shearing motion between the reticular lamina (the top surface of the cells in the organ of Corti) and the overlying tectorial membrane, deflecting the OHC stereocilia which are embedded in the tectorial membrane at their tips. The IHC stereocilia, by contrast, are not embedded in the tectorial membrane, but are deflected by the movement of fluid between the reticular lamina and tectorial membrane (Pickles & Corey, 1992). This deflection of the hair cell stereocilia places tension on the "tip links" joining adjacent rows of the stereocilia. These tip links form the gating springs for the MET channels, and this tension opens them, triggering an influx of positively charged potassium ions from the surrounding endolymph into the hair cells. This alters the intracellular voltage of the OHCs, depolarizing them and causing contraction of the cells by the action of the motor protein "prestin" (Zenner, 1988; Zheng et al., 2000). The OHC plays an active role in providing amplification of the BM movement, enhancing the sensitivity of the cochlea by up to a factor of 1000 (Kim, 1986). By contrast, in the IHCs, while the deflection of the stereocilia also leads to depolarisation, the change in electrical potential across the IHC basolateral membrane opens voltage-dependent calcium channels,

triggering the release of excitatory neurotransmitters to the primary afferent dendrites and eliciting action potentials that travel along the VIIth nerve and up the ascending auditory pathway (Hackney & Furness, 2013; Howard, Roberts & Hudspeth, 1988). Most (approximately 95%) of the afferent neurons carrying frequency specific auditory information from the cochlea to the brain are connected to the IHCs. It is the activity and tiny electrical currents along the ascending auditory pathway that give rise to the voltages that are detected and measured as the ABR.

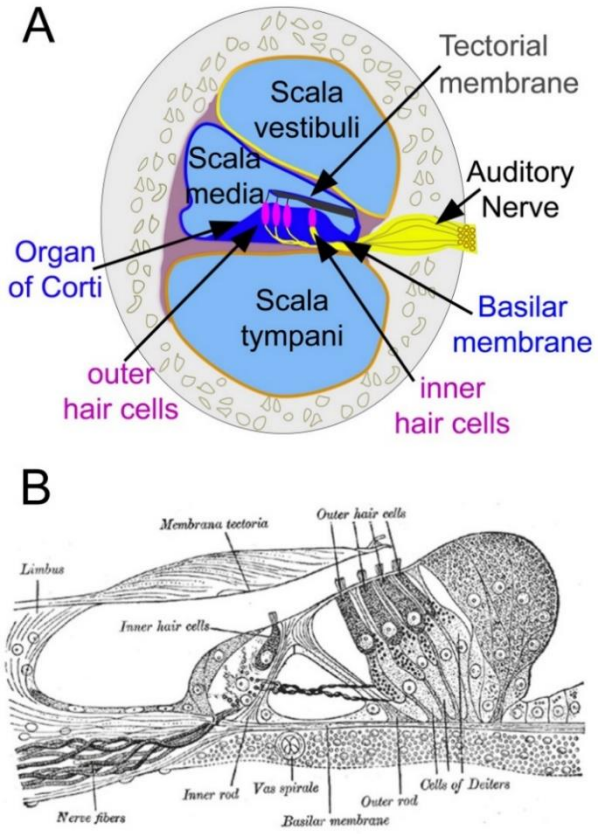

Figure 2. A. Cross section of the cochlea showing the organ of Corti and the three chambers: scala vestibuli, scala media and scala tympani. B. Diagram of the organ of Corti from Gray's Anatomy. Retrieved from "Anatomy, Head and Neck, Ear Organ of Corti", by H. J. White and D. C. Peterson, 2019, StatPearls. (https://www.ncbi.nlm.nih.gov/books/NBK538335/). Copyright 2019 by StatPearls Publishing LLC.

1.3 Auditory Brainstem Response

Auditory evoked potentials (AEP) are electrical responses generated by the nerves along the auditory pathway in response to auditory stimuli. Various AEPs can be measured depending on the underlying neural generators and timing of the electrical activity being recorded. The ABR is a far field potential arising from numerous brainstem nuclei and fibre tracts as signals travel along the ascending auditory pathway. It is a non-invasive technique that requires surface electrodes to be placed on specific locations on the scalp such as over the mastoid and forehead (Hood, 1998; Jewett & Williston, 1971).

The ABR waveform can be measured via signal averaging, whereby the recording of multiple (thousands) of measurements of the evoked potential are synchronized to the onset of the stimuli (Picton et al, 1974). The synchronized firing of the auditory neurons generates a change in the measured far-field voltage fluctuations showing as time-locked waveforms with positive peaks and negative troughs. In normal hearing participants, the waveform response yielded from the presentation of the auditory stimulus is a series of seven waves (labelled I-VII) occurring within 15 ms after the stimulus onset (Jewett, Romano & Williston, 1970; Rowe, 1978). The morphology of the ABR waveform has a characteristic shape and although interindividual variability is present, the normal response is recognizable. Jewett and Williston (1971) conducted one of the earliest ABR studies on human participants. In this study of 12 normal hearing individuals, they recorded the ABR with 60-75 dB click stimuli above the subject's threshold. They observed that the ABR waveform morphology including the peak latency were similar in all 12 subjects (Jewett & Williston, 1971).

1.4 Characteristics and Analysis of the ABR Waveform

Understanding the expected ABR response and normative peak latency from normal hearing individuals enables the observer to distinguish normal interindividual variation from abnormal patterns caused by pathology of the peripheral or central auditory system.

Alterations in the waveform vary widely depending on the pathology present as these abnormalities are often not mutually exclusive and may vary day by day.

The analysis of the presence, amplitude and latency values of the waves not only reveal information about auditory thresholds, but also about the integrity of the VIIth nerve and auditory brainstem pathway (Starr, 1976; Stockard & Rossiter, 1977; Stueve & O'Rourke, 2003; Yagi & Kaga, 1979). The latency of the peaks generally provides greater diagnostic value than the amplitude of the peaks as there is high amplitude variability among normal hearing subjects (Møller et al., 1981; Pratt & Sohmer, 1976; Thornton, 1975). Thus, techniques for categorizing waveforms are based primarily on the latencies of the components of the waveform (Star & Achor, 1975). The absolute latency of a peak is defined as the time the peak occurs after the onset of the stimulus, usually measured in milliseconds (ms). Waves I, III and V usually have absolute latency values of approximately 1.5, 3.5 and 5.8 ms respectively (Hall, 2007). The wave V peak usually appears combined with the preceding wave IV forming the wave IV/V complex. Using a relatively high intensity 70 dB HL click stimulus, a peak of 0.5 μV corresponding to wave V usually appears in adults at a latency of 5.5 - 6 ms, reached by about 12 - 18 months of age (Hall, 2007; Hecox & Galambos, 1974; Starr & Achor, 1975; Yamada, Yagi, Yamane & Suzuki, 1975). The peak latency values show the greatest transformation during early childhood and remains relatively constant from childhood and into adulthood. In one study with 42 infants, it was concluded that the latency of ABR components such as wave V decreased with maturation. This study showed that at 65 dB sensation level clicks, wave V was evoked at 9.9 ms for infants at 26 weeks gestation and this shortened to 6.9 ms at 40 weeks gestation (Starr et al, 1977).

The time differences (in ms) between peaks are referred to as interpeak latencies. These values provide information about the synchronicity and the efficiency of the conduction of the signals along different portions along the auditory pathway. The most

important interpeak latencies are between wave I to III, III to V and I to V (Hood, 1998). The IT5 is another important latency measurement, it is a measure of interaural latency difference of wave V between the right and left ears at an identical stimulus intensity. Given a client has a similar hearing configuration between both ears, it is expected that the wave V latencies should be similar meaning the IT5 value should be low equal or less than 0.2 ms (Don & Kwong, 2002).

The amplitude of a wave is determined by the voltage change (in μV) between the top of the peak and the bottom of the trough that follows it. Wave V has the largest amplitude and is usually the easiest wave to be discerned amidst biological and electrical noise, thus it tends to be used to compare latencies and as the primary indicator for the presence of a waveform. Wave V generally remains recordable in normal hearing individuals even at low intensity stimuli. Wave I and III are the other peaks of most importance for otoneurologic applications. The wave V to wave I amplitude ratio is another useful clinical criterion for neurological abnormality and is normally 1.5 while values lower than 0.5 suggests a retrocochlear pathology (Hall, 2007; Verhulst, Jagadesesh, Mauermann & Ernst, 2016).

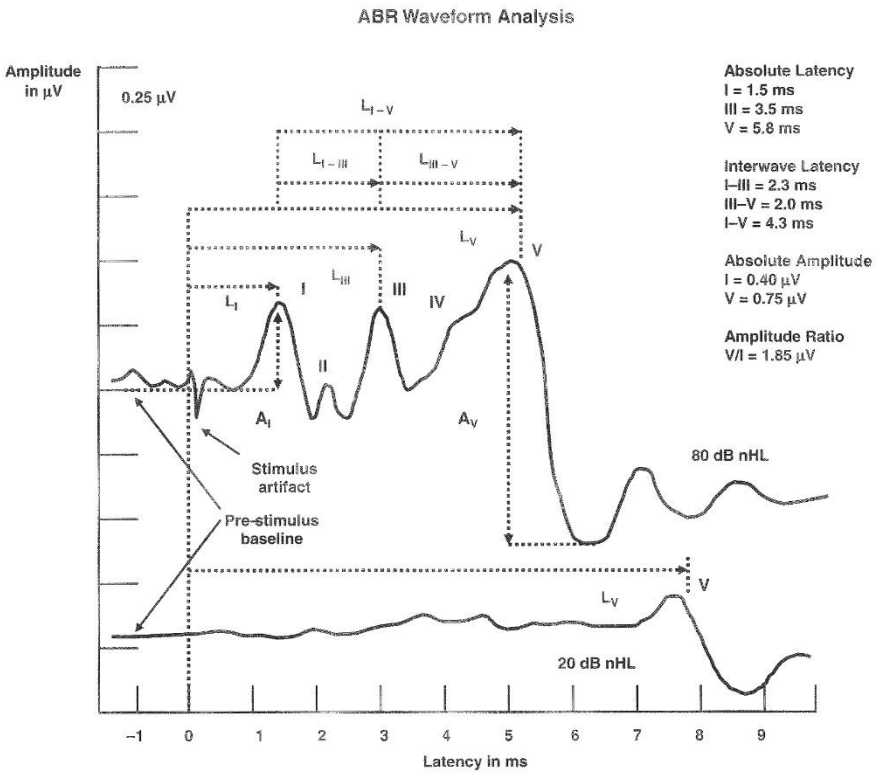

Figure 3. Example of typical ABR waveforms for a 20 and 80 db nHL stimulus, labelled with the expected absolute latency, absolute latency, interwave latency and amplitude ratio values. Adapted from "New Handbook of Auditory Evoked Responses", by J. W. Hall, 2007, Pearson. Copyright 2007 Pearson Education, Inc.

1.5 Neural Generators of the ABR

It has long been established that the vertex positive peaks of the ABR waveform are a manifestation of the activation of neural components along the auditory brainstem (Rowe, 1978). However, there has been much debate and discussion about which exact brainstem generator corresponds to which peak in the waveform. Early literature had a simplistic interpretation that each sequential peak is generated by successive sites along the brainstem. There is now strong evidence which suggests that the production of each peak is more complex, with each peak being produced by multiple generators in the brainstem. Most of the

early experiments and research on the anatomical origins of the peaks in the 1970-1980s were determined by animal experiments and human case studies with brain lesions, confirmed through imaging. Researchers also compared the latencies from recordings measured by intracranial electrodes with the latencies of recordings using surface electrodes (Achor & Starr, 1980; Jewett, 1970 Møller & Burgess, 1986; Møller & Jannetta, 1981; Møller & Jannetta, 1985; Stockard & Rossiter, 1977; Starr, 1976). Møller and colleagues conducted a range of studies to determine the origin of the waves by using both surface and needle electrodes. These results were supported by other researchers who used surface electrodes (Hashimoto, Ishiyama, Yoshimoto & Nemoto, 1981).

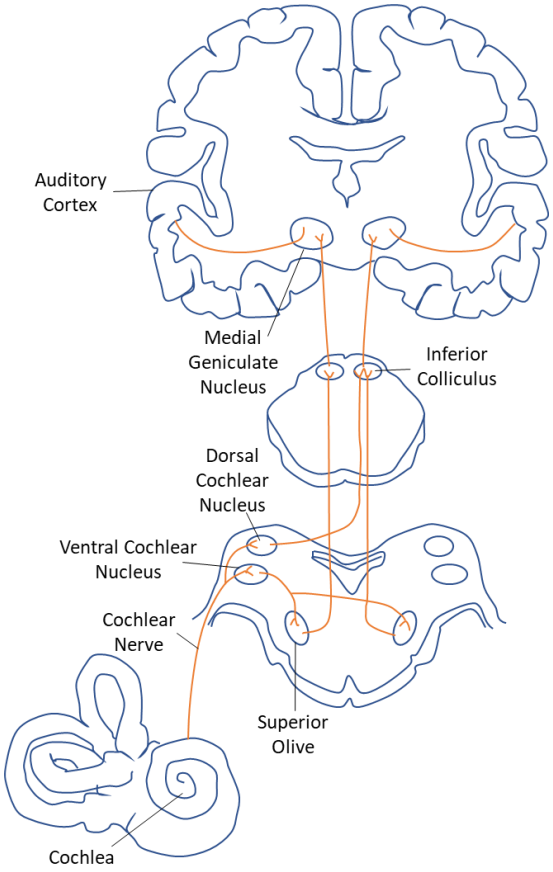

Figure 4. The ascending auditory pathway from the cochlea to the cortex including the main generators of the ABR waveform. Adapted from "Functional and structural changes throughout the auditory system following congenital and early-onset deafness: implications for hearing restoration" by B. E. Butler & S. G. Lomber. 2013. Frontiers in systems neuroscience, 7, 92. Copyright 2013 Butler and Lomber.

There is generally accepted evidence that in humans, the neural activity of the ipsilateral peripheral portion of the VIIIth nerve yields wave I of the waveform and the ipsilateral central portion of the VIIIth nerve creates wave II (Møller & Jannetta, 1981; Wada & Starr, 1983). The wave I of the ABR is the auditory compound action potential that represents synchronous VIIIth nerve activity. The VIIIth nerve terminates at the cochlear nucleus. Waves III and IV arise from the neural activity of the lower brainstem, with Wave

III generated by the cochlear nucleus and ipsilateral superior olivary complex and wave IV from multiple origins. The cochlear nucleus is the site of the first stage of central processing of auditory information and has three main divisions: the anteroventral, posteroventral and dorsal cochlear nuclei. Cochlear nerve fibres innervate different areas of the three divisions of the cochlear nucleus forming a tonotopic frequency map in each division (Rose, Galambos & Hughes, 1959). The superior olivary complex receives information from both cochlear nuclei and is divided into the lateral and medial superior olive. Together, these nuclei have roles in analysing either timing or level differences detected by both ears for localising low and high frequency sounds (Tollin, 2003). Wave V, the largest peak of the ABR, is produced by the contralateral distal lateral lemniscus and the contralateral inferior colliculus. Wave VI is generated by the contralateral medial geniculate and inferior colliculus (Hall, 2007; Hood, 1998; Lev & Sohmer, 1972; Møller et al., 1981; Starr, 1976). The inferior colliculus is an obligatory site of convergence acting as an integrative station (Schreiner & Winer, 2005).

As an example, an acoustic stimulus presented to the right ear activates the right peripheral auditory system. Signals then travel along the ipsilateral VIIIth nerve, initially in the distal portion then along the proximal portion of the nerve which corresponds to waves I and II respectively. Next the signal activates the ipsilateral cochlear nucleus and the superior olivary complex, marked by wave III of the ABR. From here, the signal spreads contralaterally as neurons from the cochlear nucleus activate the ipsilateral superior olivary complex as well as decussating to the contralateral superior olivary complex. Thus, the activity of the superior olivary complex and nucleus of the lateral lemniscus bilaterally are attributed to the wave IV of the ABR. In contrast to the other waves, wave V is produced by activity contralateral to the stimulated ear. The signals passing through the inferior colliculus and termination of the lateral lemniscus on the contralateral side produces wave V whereas the inferior colliculus on the ipsilateral side contributes very little to the wave V.

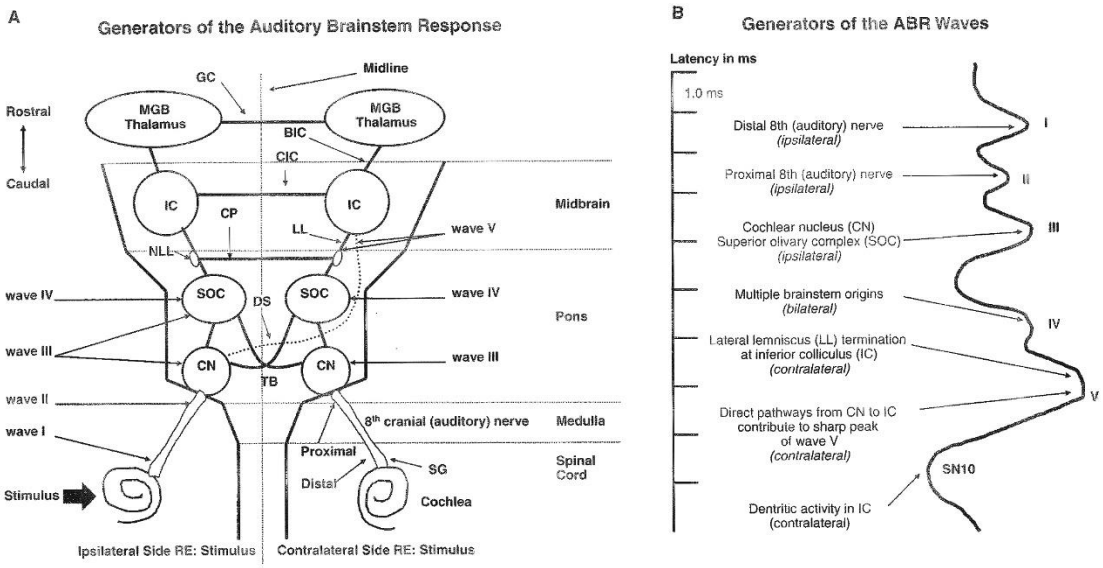

Figure 5. A. Diagram of the ascending auditory pathway in the brainstem. B. The ABR waveform with each peak labelled with the corresponding presumed brainstem generators. Retrieved from "New Handbook of Auditory Evoked Responses", by J. W. Hall, 2007, Pearson. Copyright 2007 Pearson Education, Inc.

1.6 ABR Recording Parameters

A range of recording parameters are considered when recording the ABR in order to obtain a desired response.

1.6.1 Stimulus. The most widely used stimuli to trigger the ABR response are brief transient stimuli such as broadband click stimuli or tone-burst stimuli. The ABR is a transient response meaning it is not heavily dependent on the duration or offset of the stimuli but is solely an onset response given enough time is allowed for response recovery (Hecox, Squires & Galambos, 1976). Thus, ABRs have conventionally been measured with 0.1 ms click signals. As the name suggests, broadband clicks have a broad frequency spectral content between 100

to 10,000 Hz resulting in the activation of a wide area of the BM (Gorga & Thornton, 1989; Picton, Stapells & Campbell, 1981; Stapells & Oates, 1997). Click stimuli are used to gauge the overall function of the auditory system at a wide range of frequencies rather than at specific frequencies.

While click stimuli have been used to elicit synchronous firing of the auditory neurons (Petoe, Bradley & Wilson, 2010), it is important to note the temporal dispersion of stimulus energy that occurs along the basilar membrane, as the low frequency components of the click stimuli take longer to reach the place of activation at the apex of the cochlea compared to higher frequency components that are activated earlier towards the base of the cochlea. To counteract this and increase the synchronous firing of the auditory nerve, chirp stimuli have been developed which present low frequencies first and delay the higher frequencies (Shore & Nuttall, 1985). The desired effect of this stimulus is to produce simultaneous displacement along the cochlea and for neurons that correspond to both high and low frequencies to fire simultaneously. This have been proven to show greater synchrony in neural firing, yielding a wave V amplitude about three time as large than wave V produced by click stimuli (Fobel & Dau, 2004).

Frequency specific stimuli such as a tone-bursts are used to measure hearing thresholds at specific frequencies (Bukard, 1991; Stapells, 2000). This is because the acoustic energy of a tone-burst stimulus is concentrated around a target frequency. This specificity can be achieved by increasing the rise/fall times and duration of the tone-burst and using gating windows such as the Blackman function (Gorga & Thornton, 1989; Hurley, Hurley & Berlin, 2005).

1.6.2 Electrode montage. The conventional electrode placement termed the “ipsilateral electrode montage” includes the noninverting (active) electrode being placed on the midline

on the forehead (Fz) or vertex (Cz) and the inverting (reference/indifferent) electrode on the mastoid of the ipsilateral ear being stimulated. A ground electrode is usually placed at a distant site such as the clavicle, sternum or side of the neck (Hill, 2018). Different placement configurations of the electrode arrays have been found to yield different response qualities (Parker, 1981). Studies have found that the vertical recording montage (see Figure 6) can yield significantly lower ABR thresholds, and larger wave V amplitudes compared to the ipsilateral, anterior-posterior montage which are used more commonly (Dzulkarnain, Wilson, Bradley & Petoe, 2008; King & Sininger, 1992). Dzulkarnain and colleagues recorded the ABR in 29 normal hearing adults with level-specific CE chirp stimuli, comparing ipsilateral (high forehead to mastoid) and vertical (high forehead to nape of the neck) electrode montages. Their results showed that larger wave V amplitudes were obtained with the vertical montage compared to the ipsilateral montage. Conversely, wave I and III amplitudes were significantly larger with the ipsilateral montage (Dzulkarnain, Hadi & Zakaria 2013; Dzulkarnain et al., 2017). Because the vertical montage yields larger wave V amplitudes, many studies have recommended it for recording ABR responses at lower levels and thus has high potential for threshold seeking (Sininger & Don, 1989).

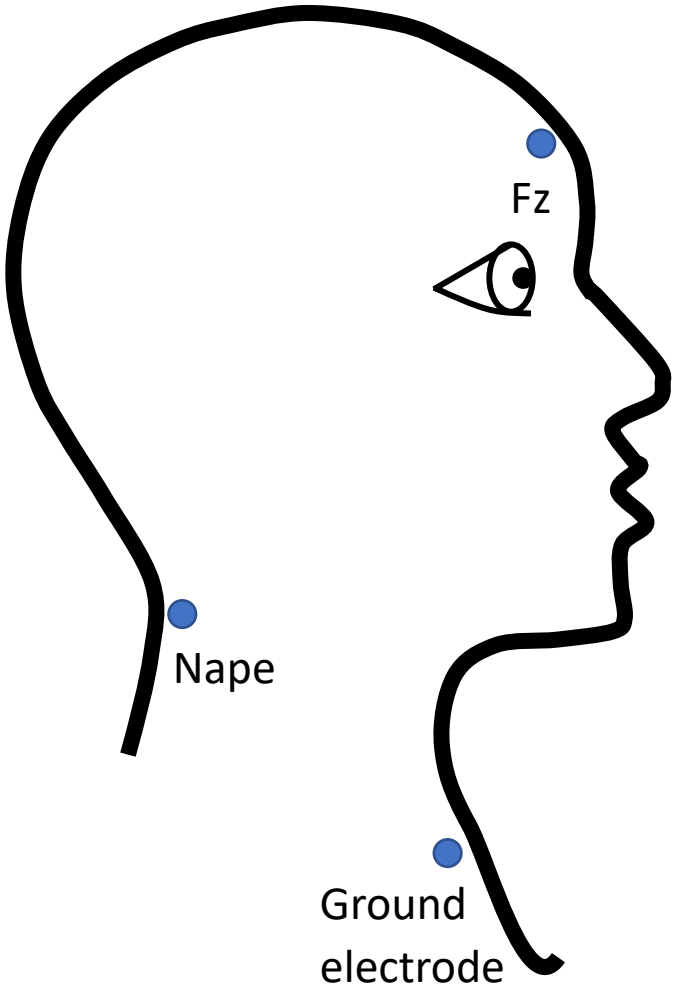

Figure 6. Electrode locations for a vertical electrode montage for recording AEPs. Fz is located on the high forehead along the midline. The nape is located in the midline, at the hairline at the back of the head. The ground electrode is placed on the clavicle.

1.7 Signal Averaging and Data Collection

The three main factors that determine the duration of an ABR test are the stimulus presentation rate, the number of different frequencies and intensity levels the stimuli will be presented at and the number of averages required. The effects of increasing the presentation rate is explored in detail in a later section. The number of different-frequency stimuli used, especially in threshold seeking is usually predetermined. Finally, the number of averages

required is chosen on the consideration of test-time and an acceptable SNR as the SNR improves the higher the number of signals are measured.

A major challenge for detecting small AEPs generated in the brainstem from a distant location on a person's scalp is being able to distinguish this signal from the background noise. Thus, maximising the SNR is a task that must be considered when measuring the ABR. The ABR is time-locked to the stimulus (Jewett, Romano & Williston, 1970). This makes time domain signal averaging a very effective method of extracting the desired signals from background noise. This involves performing numerous trials of the recording, usually presenting thousands of the acoustic stimuli and summing the signals to extract the ABR signal (Jewett & Williston, 1971). By doing so, the desired signal, although small, will be constant and summed while the random background noise that is not time-locked to the stimulus will be cancelled out, therefore improving SNR (Dawson, 1951). The presentation of these numerous stimuli and running thousands of measurements means there is a considerable time period required to obtain data for a certain frequency, at a given intensity level for one ear.

Background activity may come from numerous sources including electromyographic activity from muscles, surrounding electrical activity from electronic equipment and activity from the brain. To further increase the SNR, surrounding electrical noise from nearby mains power or other electrical equipment should be turned off or placed at a further distance from the subject. Ideally, a Faraday cage may be used to minimise and block electrical interference. Muscle movement may be a source of biological noise that could engulf the ABR signal. This can be reduced by encouraging the subject to relax and minimise movement and tension of their muscles during the test. As the ABR is not affected by sleep (Osterhammel, Shallop & Terkildsen, 1985) the participants may even be encouraged to sleep

to further minimise muscle tension. Furthermore, the appropriate application of artefact rejection will improve the averaged signals being measured (Lightfoot & Stevens, 2014).

During threshold estimation of the ABR, the "visual detection" technique involves the observer manually adjusting the stimulus level so that the evoked responses is visible on the screen amongst the background noise. The visual inspection of the averaged result is a subjective measure of the SNR and this subjective element requires knowledge and experience from the observer to yield accurate estimation of thresholds. Alternatively, objective automatic detection is favoured when visual interpretation is difficult due to poor SNR. Objective methods in determining the presence of the ABR are mostly used in screening procedure especially in the paediatric population. This minimizes the variability inherent in subjective, visual interpretation of the ABR. The detection formula used in automatic ABR requires an estimation of the SNR to determine a response amongst the background noise (Elberling & Don, 1987). An objective detection method described by Elberling and Don (1984), uses variance analysis to calculate the ratio between the magnitude of the ABR compared to the averaged background noise. This value is termed the Fsp and can be set at a desired value which determines the quality of the waveforms obtained and the length of testing (Elberling & Don, 1984). A follow up study was able to demonstrate that using a Fsp value of 3.1 is equivalent to an SNR of 2.1 and is a viable quantitative method in the automatic threshold detection of the ABR. An Fsp of 3.1 also yields a low rate of false positive at 1%. Although the Fsp does not provide direct information on the latency and amplitude of the waveform components, this method quantitatively determines that the ABR obtained is of sufficient quality in terms of the SNR. Clinically, this is valuable for threshold estimation where the statistical presence of a waveform is of primary importance and the actual amplitude and latencies of the waveforms are less important (Elberling & Don, 1984). Other automatic tracking methods determine the threshold responses based on the correlation

between successive or interleaved responses as well as statistical test procedures in the frequency domain (Berninger, Olofsson & Leijon, 2014; O'Beirne, 2005).

1.8 Auditory Brainstem Response in Clinical Practice

The three main clinical applications of the ABR are for predicting audiometric thresholds, differential diagnosis of retrocochlear pathologies and intraoperative monitoring. The ABR is an invaluable clinical tool because it is a non-invasive technique that provides objective diagnostic information for adults and children who are unable to perform behavioural audiometric testing reliably (Coles, 1997; Hood, 1998; Stapells & Oates, 1997). The ABR has become a staple component in the audiological test battery for universal newborn hearing screening protocols. With ABR recordings, click stimuli may be used to obtain estimates of hearing function in a screening protocol but in comprehensive testing, tone-burst stimuli are often used to obtain frequency specific estimates of thresholds. Because background noise must be reduced to obtain robust responses, children may be sedated in order to minimize movement and noise from muscular artefacts. The application of this method is usually accurate and predicted thresholds are usually within 10 dB of behavioural thresholds (Hood, 1998).

Comparisons between bone conducted and air conducted ABRs may be used to determine if the cause of the hearing loss is sensorineural or conductive. Latency-intensity functions of the ABR may also indicate the type of hearing loss. Sensorineural hearing losses are characterized by a steeper latency-intensity curves, falling out of normative range at lower intensity levels. Comparatively, conductive hearing losses attenuate sounds before reaching the cochlea, and therefore all latencies are prolonged at all stimulus intensities and a shift of the curve to the right is observed (Atcherson & Stoody, 2012; McGee & Clemis, 1982). The interleaved paradigm described in this study has the potential to be a useful tool in creating intensity-latency functions as further explained in section 4.4.

Variations in ABR responses can be used to diagnose neurological disorders as certain features of the response are correlated with various pathologies and site of lesion along the auditory brainstem. The latency values measured may be compared to normative absolute and interwave latency values (Hood, 1998). For example, the standard criteria used for diagnosing a tumour with an ABR are prolonged absolute wave V latency and interpeak latencies. In conjunction with other diagnostic tests and imaging procedures, ABR responses are used as a diagnostic criteria for determining neurological pathologies along the auditory brainstem such as VIIIth nerve tumours and focal lesions and auditory neuropathy spectrum disorder (Madden et al., 2002; Sohmer, Feinmesser & Szabo, 1974; Starr, 1976; Starr et al., 1996). There are characteristic patterns which suggest if the hearing loss present has a conductive, sensorineural or neural origin. While magnetic resonance imaging (MRI) has become the gold-standard for detection of VIIIth nerve tumours, ABRs are more readily available, less cumbersome to use and may be cheaper overall. The convenience factor that the ABR offers makes it a valid alternative over scanning procedures that may be more diagnostically accurate but have a significantly limited availability.

During surgical procedures involving the auditory or vestibular system, the hearing ability of the patient may need to be measured in short intervals while the procedure is being performed. This is to determine if the procedure has restored auditory function prior to completing the procedure as well as to monitor the preservation and prevention of trauma to the auditory nerve. The ABR provides a means to provide a measure of auditory function with minimal interference to the surgical procedure being done (Ren et al., 2017).

1.9 Comparing the Adult and Newborn ABR

Age is a key subject characteristic that influence AEP recordings and there are key differences in the adult ABR responses compared to paediatric populations. The cochlea may be fully functional by about 35 weeks conceptional age but the features of newborn ABR

continue to undergo notable maturational development over the first two years of life. The ABR peak latencies of newborns are longer than in adults and younger individuals are expected to have longer ABR peak latencies as shown in Figure 7. Newborns and young individuals have immature and developing neural systems. Complete myelination and optimal synaptic efficiency may not have yet been achieved. This leads to longer transmission times for signals travelling through the auditory pathway and this is reflected as prolonged latencies of ABR components (Lasky 1984; Pratt & Sohmer, 1976). As a newborn ages, normal neurological development allows acoustic signals to be transmitted more efficiently and the ABR latencies are expected to progressively decrease closer to average adult values. The peripheral auditory system exhibits maturation earlier than central components of the auditory system hence why the wave I amplitude in newborns may be larger than other wave components (Stockard, Stockard & Coen, 1983). Additionally, interlatency values are greater and latency values for later ABR waves are usually prolonged in newborns compared to adults (Eggermont & Salamy, 1988).

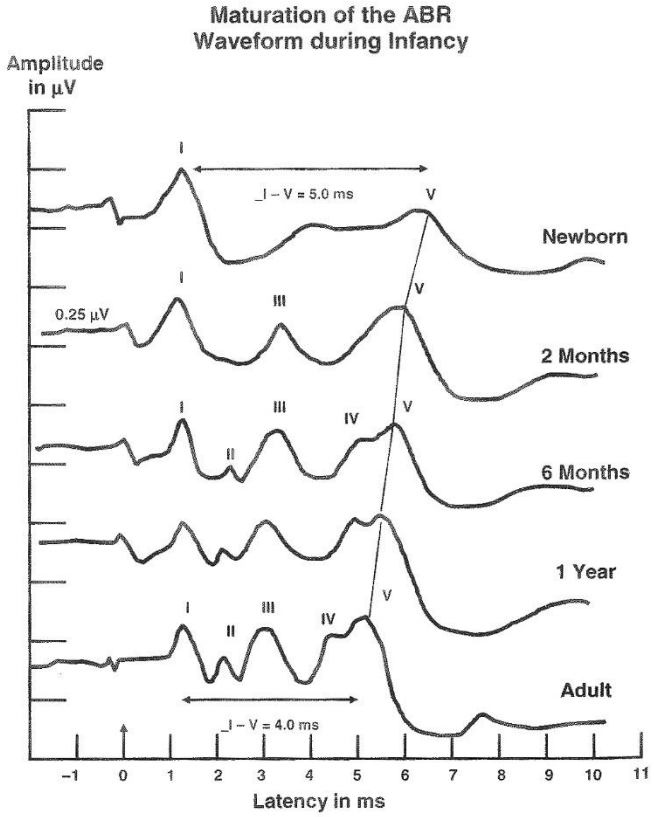

Figure 7. Maturation of ABR waveform morphology from newborn to adulthood. Adapted from "New Handbook of Auditory Evoked Responses", by J. W. Hall, 2007, Pearson. Copyright 2007 Pearson Education, Inc.

Observers must take age into consideration when interpreting ABR responses of children especially under 18 months old. Significant maturation effect of the ABR are observed when using click stimuli from birth until about two years of age. Numerous studies have studied morphological changes in the ABR of children in the early stages of life. Hurley and colleagues (2005) tested 305 infants with ages ranging from 33 to 74 weeks conceptional age to investigate maturation effects using low frequency tone-bursts. The trend reported was a major decrease in wave I, III and V latency with age when click and 500 Hz tone-bursts were used at 25, 35 and 55 dB nHL. This trend did not cease by 70 weeks conceptional age

(Hurley, Hurley & Berlin, 2005). These results were consistent with previous studies which observed a progressive decrease in wave V latency until about 18-24 months of age post birth. One of these studies include a large investigation of 585 physiologically stable babies who graduated from an intensive care nursery. The wave V latency decreased with age and was observed to stabilize by 23-24 months of age (Gorga et al., 1987). Ponton et al. (1992) generated similar results in their study and showed that the wave V latency stabilized at a similar age. Furthermore, their study concluded that development and maturation occurred faster and earlier for ABRs corresponding to mid frequencies. This contrasted previous studies which showed that the maturation for peak and interpeak latencies occurred earlier for responses stimulated by low frequency stimuli (Ponton, Eggermont, Coupland & Winkelaar 1992).

The differences in the effects of rate on newborn ABR compared to the effects on rate on adult ABR has also been studied. The reported interaction of age with high stimuli rates between studies appear to be mixed with some studies reporting similar responses between adult and paediatric population while some showing evidence for different responses between the two groups. Burkard & Sims (2001) investigated aging effects on the ABR wave V latency at click rates of 11, 25, 50 and 75 Hz. It was reported that the changes in ABR with increasing rates were similar between the young adult and older adult groups when both groups had normal hearing thresholds (Burkard & Sims, 2001). Pratt et al. (1981) conducted a study comparing 10 adults and 10 children who were audiometrically normal, presenting high intensity click stimuli at 10/s and 50/s. From the lower to the higher stimuli rate, a wave V latency shift of 0.35 ms was observed for both groups (Pratt et al., 1981).

On the contrary, some studies have concluded that increasing the repetition rate produced a greater wave V latency shift in neonates compared to adults. Lasky (1997) conducted a series of experiments comparing rate effects in the ABR of newborns and adults,

see Figure 8. The results from this paper included newborn and adult latency/ rate functions and showed that wave II and V latency/rate effects were significantly greater for newborns than in the adult group. Wave I latency/rate effects were similar. Interestingly, adults exhibited a greater reduction in amplitude with increasing stimulus rate compared to newborns (Lasky, 1997). The incomplete maturation of the central nervous system in newborns is considered to be the reason for the greater wave V latency shift observed in newborns (0.75 - 0.9 ms) compared to adults (0.3 - 0.5 ms) in response to higher stimulus rates (Parthasarathy, Borgsmiller & Cohlan, 1998).

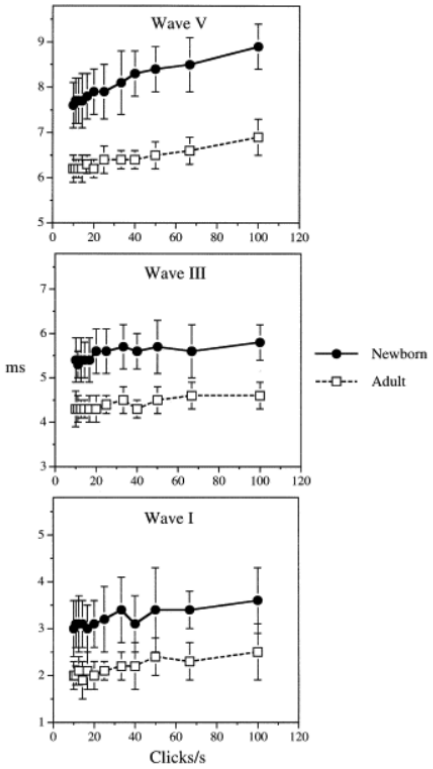

Figure 8. Figure showing the effect of stimulus rate on the latency of waves I, III and V of newborn and adult ABR. Retrieved from "Rate and adaptation effects on the auditory evoked brainstem response in human newborns and adults" by R. E. Lasky. 1997. Hearing research, 111(1-2), 165-176. Copyright 1997 Elsevier B.V.

1.10 Effect of ABR Stimulus Intensity

The latency and amplitude of an ABR waveform are affected by stimulus properties such as frequency, intensity and rate (Hambley, 2014; Stone et al., 2009). In a healthy auditory system, when a click is presented at an intensity above the threshold, the clicks evoke synchronous activity of the neurons in the auditory pathway resulting in visible wave peaks. The amplitude and latency of the ABR response varies with stimulus intensity. Clinical research and testing demonstrate that the peak-to-peak amplitudes increase, and absolute peak latencies decrease, the higher the intensity of the stimulus being presented (Hecox & Galambos, 1974; Hood 1998).

Generally, with a high intensity stimulus, wave V appears at about 5.5 ms and has an amplitude of 0.5 µV and this value rarely exceeds 1 µV even at very high intensities. At higher stimuli intensities, a greater number of nerve fibres are recruited, therefore a larger electrical signal along the auditory nerve is being detected shown as larger peak amplitudes (Stypulkowski & Van den Honert, 1984). Thus, high intensity clicks are an appropriate choice for otoneurologic applications as it develops clear ABR peaks for assessment of the auditory pathway up to the inferior colliculus as marked by a well-defined wave V peak. Conversely, as the intensity is decreased, the amplitudes steadily decrease until the ABR waveform becomes absent near the person's auditory threshold. This relationship is what is utilized to determine peripheral acuity. A study by Picton et al. (1981) observed a reduction in wave V amplitude from 0.6 to 0.35 µV as the stimulus intensity was decreased from 80 to 30 dB nHL. Although the wave V peak is the clearest component when identifying peaks, waves I and III also demonstrate these effects with varying stimulus intensity and have even been shown to have an even greater reduction in amplitudes at lower stimuli intensities (Jewett & Williston, 1971; Picton et al., 1981).

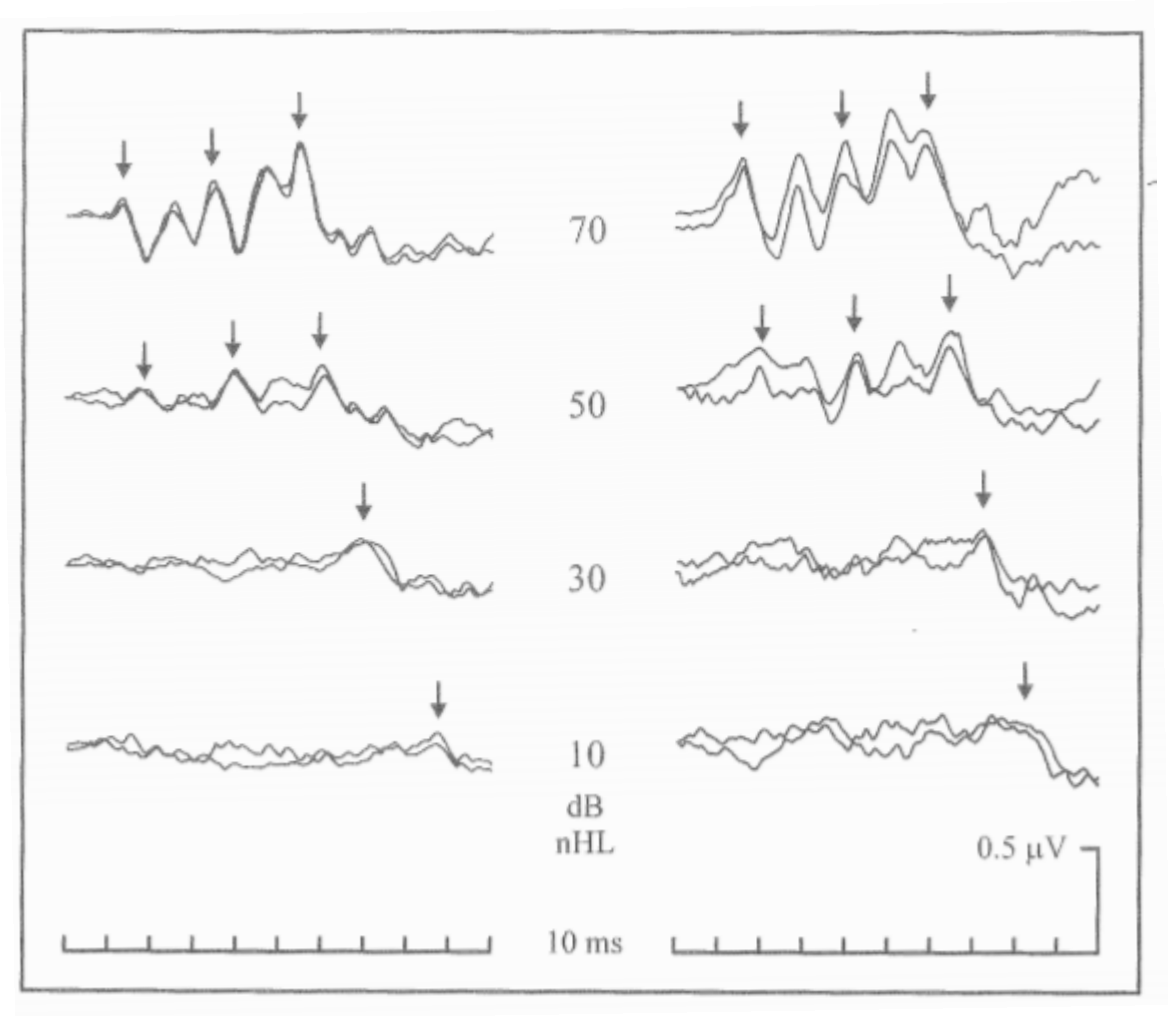

Figure 9. Effect of stimulus intensity on ABR waveforms with click stimuli at a rate of 11/s (left) and 20/s (right). Adapted from "Human auditory evoked potentials", by T. W. Picton, 2010, Plural Publishing. Copyright 2011 Plural Publishing, Inc.

However, the increasing trend in latency as the stimulus intensity is decreased, is not linear. The greatest change in latency is observed at lower intensities as plotted in a typical ABR latency-Intensity Function (Hecox & Galambos, 1974). One of the proposed explanations for the latency-intensity relationship is that the postsynaptic excitation potentials reach threshold faster for higher intensities and together these create a latency difference of about 2.5 ms from low to high intensity (Picton et al., 1981).

1.11 ABR Stimulus Rate

The stimulus rate may be adjusted to allow for more rapid data acquisition. Generally, rates up to 20/s have little effect on the ABR however, higher rates can have significant effects on the waveform morphology, especially to waves I and III. Hence, there is a consideration to be made between obtaining data as fast as possible but still obtaining robust ABR morphology (Petoe, Bradley & Wilson, 2009). ABR systems that are commercially available are usually capable of presenting click stimuli at rates of 9-99/s but conventionally, stimuli rates of 9.1- 33.3/s are used as they result in optimal resolution of waves I, III and IV (Leung, Slaven, Thornton & Brickley, 1998; Petoe, Bradley & Wilson, 2009). Rates of up to 25- 40/s are also considered acceptable (American Speech-Language-Hearing Association, 1987). Paludetti, Maurizi & Ottaviani (1983) noted similar waveform morphology between 10 – 20 clicks/s and reported only minor changes at 50 clicks/s. However, at 100 clicks/s a clear decrease or disappearance of some wave components were observed (Paludetti, Maurizi & Ottaviani, 1983). For this thesis, because high stimulus rates were investigated using the interleaved paradigm, it is of interest to explore and discuss established literature regarding the effects of the application of high stimulation rates. This guided the selection of appropriate rates to trial and enhanced understanding of what to expect at high stimulus rates and the mechanisms of these effects.

A highly cited paper by Gerling and Finitzo-Hieber (1983) collected normative data on 48 normal hearing adult subjects. This normative data was then compared to the ABR results of 221 patients, 131 individuals had normal hearing and 90 had impaired hearing sensitivity. The high stimulus rate ABR at 90 clicks/s was the only parameter effective at identifying patients with central nervous system (CNS) pathology from having prolonged wave V latency. This paper is one of the earliest studies to recommend the routine use of high stimulus ABR to aid in the diagnosis of brainstem pathology (Gerling & Finitzo-Hieber,

1983). Sturzebecher and colleagues (2003) reviewed a fast screening algorithm on high click rate ABR. The automatic detection was carried out by a statistical test procedure in the frequency domain. Repetition rates from 20 – 400 clicks/s were tested first on 25 adults and it was determined that a rate of 140/s lead to a mean detection time of 31s and was the optimal rate for quickly detecting ABR for a screening protocol in the adult group. For a group of 114 neonates, a click rate of 90/s was the optimal rate under their proposed detection algorithm (Sturzbecher, Cebulla & Neumann, 2003). Note that in these cases, the aim is *detection* of the ABR, rather than the preservation of wave morphology. Many studies have investigated the characteristics of alternative types of stimuli presented at rapid rates, such as maximum length sequences (MLS) stimuli, which have been shown to evoke ABRs at very rapid rates up to 854 Hz (Burkard, Shi & Hecox, 1990; Burkard, 1994; Eysholdt & Schreiner, 1982; Li et al, 1988), and which use a deconvolution process to extract the ABR waveform.

Along with minimising test time, some research has proven that there is also diagnostic value with conducting ABR with high stimulus rates. It has been reported that the response abnormalities due to high stimulus rates are useful indicators of acoustic neuroma (Daly, Roeser, Aung & Daly, 1977). Tanaka, Komatsuzaki and Hentona (1996) assessed the ABR of 40 patients with acoustic neuroma, 42 patients with normal hearing and 30 patients with sensorineural hearing were recorded at stimulus rates of 9.5, 20, 40 and 90 clicks/s. They observed a significantly greater interpeak latency between wave I and wave V at each stimulus rate in patients with acoustic neuroma compared to control groups. They concluded that recording ABR at high stimulus rates provides useful information in detecting acoustic neuroma patients with normal ABRs (Tanka, Komatsuzaki & Hentona, 1996). A study by Ackley and colleagues (2006) used rapid stimuli rates up to 61.1 clicks/s on 20 otologically normal participants. They concluded that with these rapid stimuli levels, using a wave V latency of 61.2 ms as an appropriate lower limit for referrals of will be sufficiently sensitive

to subtle neurological defects. Employing this procedure allowed their group to correctly identify a patient in the study with a small vestibular schwannoma that would not have otherwise been identified using standard ABR protocols (Ackley, Herzberger-Kimball, Burns & Balew, 2006). High click stimuli rates in ABR were also studied in multiple sclerosis patients with normal MRI to determine if performing high click rate ABR improves diagnosis. Results by a paper from Santos and colleagues (2004) concluded that the inclusion of stimulus rates of 51 and 61 click/s in ABR testing may be of diagnostic value with demyelinating diseases such as multiple sclerosis. The results of their study show that the absolute latencies at these high frequencies were significantly greater in patients with multiple sclerosis (Santos, Munhoz, Peixoto & Silva, 2004). These recommendations for the use of high rate stimuli for diagnostic use are predicated on the rationale that the short recovery times with high stimulus rates causes major delays in wave V latency especially when the auditory nerve is compromised.

Overall, the literature on the use of high stimulus rate for ABR testing is extensive but the suggested optimal rates vary depending on the framework and protocols of testing and the aims of each respective research groups. However, there are a few factors that should be considered when deciding what stimulus rate should be used with the proposed interleaved method. Generally, for very brief transient stimuli, the accumulated time of the actual stimulus is considered negligible and the interval between each successive stimulus can be calculated by dividing the length of time by the number of stimuli presented within that time period. In other words, the interstimulus interval (ISI) is the time between each adjacent stimulus. For example, if a transient click stimulus is presented 20 times in 1000 ms then (1000 ms/ 20 = 50 ms) the ISI is 50 ms. This means that at very high stimulus rates, there is very short ISIs between each successive stimulus. Thus, the recovery period for the neurons becomes shorter. Technically, the maximum rate an evoked response can be activated is

restricted by the response time length, also known as response time epoch. The duration of a human ABR response is at least 10 ms so the maximum rate of stimulation is 100 Hz, before each successive response overlap each other (Burkard, 1994).

Despite many promising procedures and research on rapid rate ABR and many researchers suggesting the routine use of high stimulus rate ABR for its diagnostic value, currently the use of rapid stimulus rates for neurological diagnostic assessment is not widespread.

1.12 Effects of Stimulus Rate on ABR

Multiple studies have addressed the effect of increasing the stimulus rate on the ABR waveforms. As shown in Figures 8 and 10, the effects observed in a normal hearing system are that as the stimulus rate is increased, the ABR wave peak latencies increases and wave amplitude decreases. At very high stimulus rates the waveform morphology loses definition (Don, Allen & Starr, 1977; Fowler & Noffsinger, 1983; Gerling & Finitzo-Hieber, 1983; Hyde, Stephens & Thornton, 1976; Jewett & Williston, 1971; Pratt & Sohmer, 1976; Leung, Slaven, Thornton & Brickley, 1998; Weber & Fujikawa, 1977). In a study of 6 normal hearing adults, Don, Allen & Starr (1977) found that at 60 dB SL the mean V latency increased from 5.95 ms at a rate of 10/s to 6.30 at 30/s, 6.38 at 50/s and 6.60 at 100/s. (Don, Allen & Starr, 1977). This finding was supported by Paludetti and colleagues (1983) who also observed a 0.5 ms wave V latency shift between 10 to 100 clicks/s at intensities of 60 and 70 dB nHL in their study of 26 normal hearing subjects (Paludetti, Maurizi & Ottoviani, 1983). A similar wave V latency shift of 0.61 ms with a standard deviation of 0.14 ms was observed between a rate of 20 and 90 clicks/s at 60 dB nHL for 48 normal hearing subjects. It was noted that the criterion for an abnormal latency shift is a shift greater than 3 standard deviations above the mean (>1.04 ms) (Gerling & Finitzo-Heiber, 1983). Another study by Weber & Fujikawa (1977) recorded the ABR from 22 adults at 60dB SL and observed wave

V latencies of 5.84, 5.9 and 6.18 ms at 13.3, 33.3 and 67 clicks/s respectively. A clear deterioration in waveform clarity and reduction in wave amplitudes were also reported (Weber & Fujikawa, 1977). A study by Parthasarathay, Borgsmiller and Cohlan (1998) measured the ABR in 10 adults and 10 neonates who have normal hearing and used high intensity tone-bursts at rates of 11.1 and 55.5/s. They found that increasing the stimulus rate prolonged the wave V latency for both adults and neonates (Parthasarathy, Borgsmiller, & Cohlan, 1998). Generally, for every increase of 10 clicks/s there is a shift in wave V latency of approximately 0.1 ms (Daly, Roeser, Aung & Daly, 1977). Valderrama and colleagues gathered data on the adaptation mechanisms on ABR at high stimulus rates. The results from this study again showed that both amplitudes and latencies of the ABR are influenced by fast and slow adaptation mechanisms at high stimulation rates. Their results showed significant wave III and V amplitude decrease and latency increase when using stimuli with short ISIs (2-5ms) compared to stimuli with long ISI (21-24ms) (Valderrama et al., 2014).

The ABR wave components interact differently to high stimulus rates. Studies have shown that the wave I latency is more resistant to higher stimulation rates compared to wave V which apparently displays more effects of neural fatigue at high stimulation rates. Picton et al. (1981) showed that with an increase of stimulus rate from 10 to 80 clicks/s, the latency of wave I only increased by about 0.14 ms whereas the latency of wave V increased by about 0.39 ms. With a change in rate from about 20 to 80 clicks/s the wave V latency shift have usually been ~0.4 – 0.6 ms but can be as low as 0.25 ms which is still greater than expected wave I latency shifts at the same rate range (Burkard & Sims, 2001; Gerling, 1989; Picton et al., 1981; Pratt & Sohmer, 1976; Stone et al., 2009; Weber & Fujikawa, 1977; Yagi & Kaga, 1979).

In contrast, in terms of wave amplitude, wave V shows less reduction than the earlier waves I and III which are more affected by increasing rates. From a slow stimulus rate of

around 10 clicks/s to a rapid rate of 80 clicks/s, wave I exhibits a reduction in amplitude by 50% whereas the wave V amplitude decreases by about 10-30% of the original amplitude. These findings are believed to be explained by the overlapping convergence and divergence of neurons in the ascending auditory pathway (Hall, 2007; Picton et al., 1981; Pratt & Sohmer, 1976). While, waves I, II and IV may be less indistinguishable or even disappear at very high presentation rates above 80/s waves III and V tend to remain visible even at high stimulus rates. In normal hearing adults, a range of mean amplitude reductions of wave I amplitude between 10-38% were observed when increasing the stimulus rate from 10 to 20/s. Wave I reduces further by 39-50% when the stimulus rate is increased to 50/s from 10/s (Lasky, 1984; Mouney et al., 1978; Pratt & Sohmer, 1976; Scott & Harkins, 1978; Zollner, Karnahl & Stange, 1976). In comparison, increasing the stimulus rate from 10/s to 50/s appears to have less effect on wave III only showing a 30% reduction in amplitude (Hyde, Stephens & Thornton, 1976). Interestingly, the wave V amplitude initially increases with increasing presentation rates reaching maximum amplitudes at rates around 40 to 50/s then gradually decrease at higher rates (Lightfoot, 1992; Paludetti, Maurizi & Ottaviani, 1983; Scott & Harkins, 1978). The wave V amplitude decreases by about 10 % to 30 % at high stimulus rates compared to original amplitudes at lower stimulus rates (Hall, 2007).

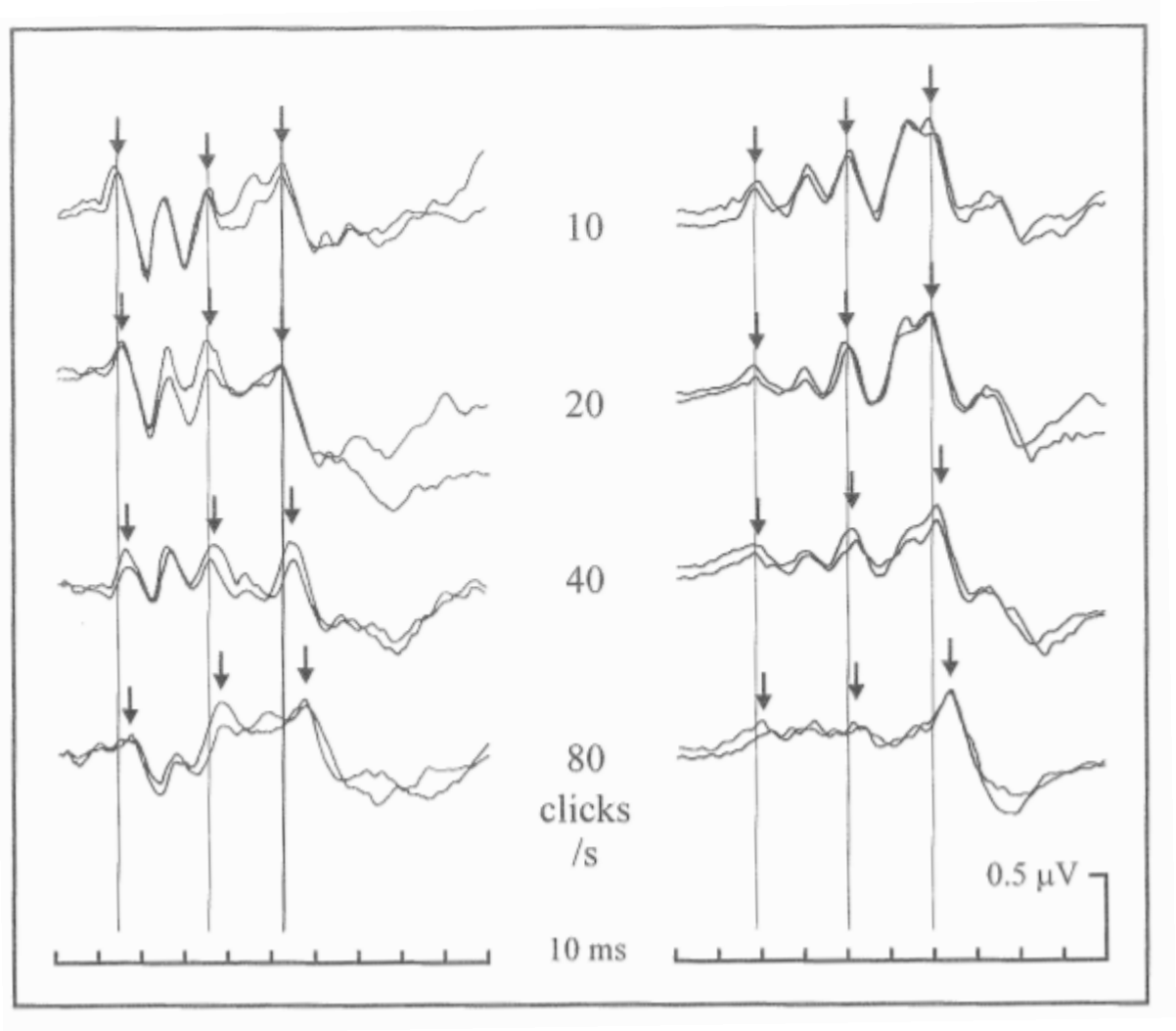

Figure 10. Effect of stimulus rate on ABR latency, amplitude and morphology with click stimuli at 70dB nHL (left) and 60dB nHL (right). Adapted from "Human auditory evoked potentials", by T. W. Picton, 2010, Plural Publishing. Copyright 2011 Plural Publishing, Inc.

1.13 Adaptation and Fatigue

These changes in response at higher stimulus rates impose a limit to how fast a stimulus rate may be increased. The rate-induced increase in peak latency and reduction in wave V amplitude is believed to be a result of neural adaptation and fatigue that is observed

at higher stimulus rates. The prolonged latency and reduced response clarity observed in high stimulus rate may also be attributed to the desynchronised neural firing.

The adaptation of the auditory nerves can be quantified by the deterioration in response (i.e. decreased wave amplitude and increased peak latency) due to successive click stimuli compared to the preceding stimulus (Thornton & Coleman, 1975). Immediately after a neural event such as an action potential (AP) there is a brief period during which the neural unit is either incapable of being activated or has a higher threshold of activation (Hall, 2007). Many proposed models of adaptation have been presented. Proposed explanations for the neural adaptation effect include an increase in synaptic inefficiency or the depletion of neurotransmitter reservoirs (Don, Allen & Starr, 1977; Pratt & Sohmer, 1976; Smith & Brachman, 1982). The transmission of signals between neurons require the release of neurotransmitters between each neuronal junction and if these chemicals become exhausted during high stimulation rates then the transmission of these signals become degraded. If the ISIs are long enough to exceed the recovery period, then the neurons will have sufficient recovery to fully respond to the next stimulus. However, if the ISIs are shorter than the recovery period then a suboptimal response is recorded as the neural units may not have fully recovered before being activated by the next stimulus. Thus, as the ISI is decreased, the amount of adaptation increases (Thornton & Coleman, 1975). As explained above, this adaptation is reflected as a change in response such as increased peak latency or decreased amplitudes. The refractory period in auditory neurons are relatively quick, lasting one to a few milliseconds (Avissar, Wittig, Saunders and Parsons, 2013; Van den Honert & Stypulkowski 1984). Thus, in order to evoke quality ABR responses, very brief ISIs are sufficient and subsequently relatively high rates of stimulation is permissible. A detailed discussion regarding peripheral versus central rate adaptation effects is presented in Chapter 1.16.

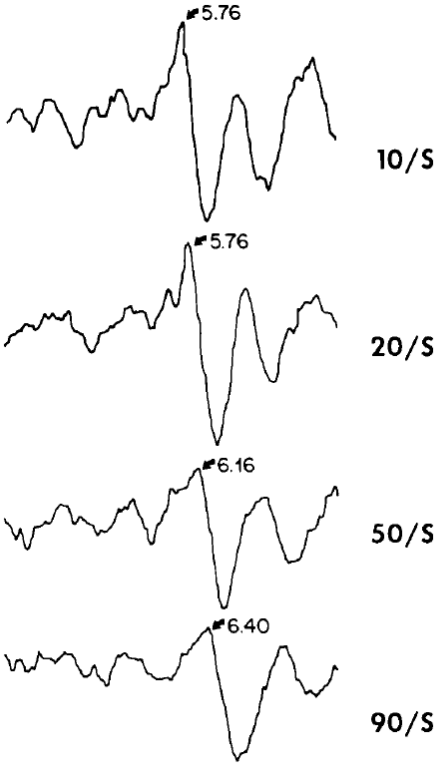

Figure 11. ABR waveform responses showing wave V peak amplitude and latency differences across four stimulus rates for a typical subject. Retrieved from "Auditory brainstem response with high stimulus rates in normal and patient populations" by I. J. Gerling & T. Finitzo-Hieber. 1983. Annals of Otology, Rhinology & Laryngology, 92(2), 119-123.

The cochlear nerve compound activation potential in electrophysiological recordings are denoted as N_1. Sørensen (1959) studied auditory adaptation in nerve action potentials of guinea pigs and proposed that the adaptation of the cochlear nerve compound action potential and subsequently the decrease in neural activity recorded was the result of either 1) the decrease in the number of active nerve fibres while the firing rate of the active nerves remain constant or 2) the number of active fibres remaining constant but the firing rate of all these fibres decreasing (Sørensen, 1959). Eggermont & Spoor (1973) conducted further study on

the cochlear adaptation in guinea pigs many years later and their results supported the second mechanism from Sørensen's paper (Eggermont & Spoor 1973). A paper by Thornton & Coleman (1975) presented several adaptation models of the auditory system. They tested the adaptation of cochlear nerve and auditory brainstem components. The results best fit one of their proposed models which was that adaptation occurs at all levels of the auditory system but because there are different levels of adaptation at each stage ($N_1 – N_5$). Potentially, N_1 and N_2 adapt as per Sørensen's first mechanism of adaptation while the following responses follow Sørensen's second mechanism.

Overall, there are numerous different adaptation models proposed in literature, but the consensus principle is that high rate stimuli stress the auditory system by decreasing recovery period and maximising the rate at which the neurons can effectively transmit signals.

1.14 ABR vs ASSR

The auditory steady state response (ASSR) is another AEP that is predicated and measured on similar principles and recording set up as the ABR, but with some important modifications. The ASSR records a larger and latter part of neural activity along the auditory pathway than that measured in the ABR. It also provides information regarding the integrity of subcortical areas. The ASSR is obtained by sinusoidally amplitude-modulating the carrier tone of interest (for example, 500 Hz if probing the low-frequencies, or 4 kHz for the higher ones) at approximately 40 Hz, hence why the ASSR is also termed the "40 Hz response" (Galambos, Makeig & Talmachoff, 1981). The modulation at 40/s causes the positive and negative peaks of the responses to overlap at 25 ms intervals thereby reinforcing the auditory middle-latency response (AMLR). Other modulation frequencies may be used for example, between 60-100Hz. The 90Hz ASSR elicits a response from the same components of the auditory brainstem responsible for the ABR (Cohen, Rickards & Clark, 1991; Kraus et al.,

1994, Rickards et al., 1994). The continuous amplitude-modulated tone used to evoke the ASSR is more frequency specific (i.e. has less spectral splatter) than transient stimuli such as tone bursts.

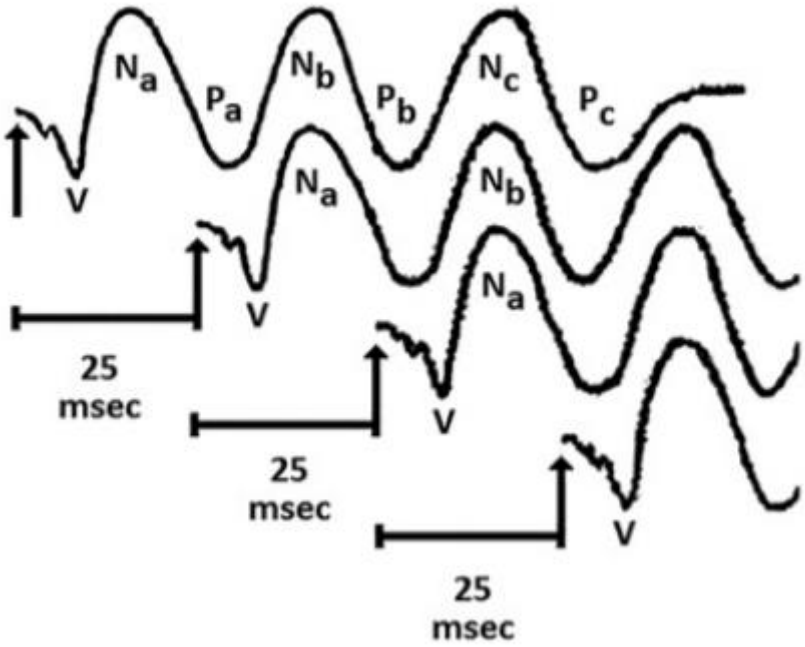

Figure 12. Diagram showing how the peaks of multiple ABR and MLR waveforms would overlap at 25 ms intervals to produce the 40 Hz ASSR. Retrieved from "Auditory steady-state responses" by P. Korczak & T. Finitzo-Hieber. 2012. Journal of the American Academy of Audiology, 23(3), 146-170.

It is possible for multiple ASSRs to be evoked at the same time by using multiple frequency specific stimuli to stimulate different regions along the BM and thus different populations of neurons from the same ear simultaneously. This concept was first demonstrated by Regan & Regan (1988) who used two tones with different modulation frequencies (Regan & Regan, 1988). The multiple auditory steady-state response (MASTER) technique employs an automatic statistical evaluation of multiple amplitude and/or frequency

modulated stimuli of the ASSR and displays these in the frequency domain. It then quickly determines if these responses are valid in comparison to background noise (John & Picton, 2000). This procedure can be applied to assess hearing at different frequencies simultaneously which reduces test time as multiple recordings can be completed in the time usually required for one (Dimitrijevic et al., 2002; John et al., 1998). Even though multiple ASSR recordings can be performed bilaterally at multiple frequencies, obtaining results at multiple intensities still have to be done sequentially. These responses, like the ABR can be used as an objective measurement to assess auditory acuity in adults and children and this is accomplished by identifying the ASSR response based on their amplitude and phase shift using Fourier analysis (Galambos, Makeig & Talmachoff, 1981; Rance & Rickards, 2002).

A paper by Cone-Wesson and colleagues (2002) reported two studies comparing the ASSR and the ABR. The first study was a retrospective review of 51 cases and found robust correlations in the thresholds of both methods while the second study provided evidence that the automatic detection algorithm was a reliable tool for threshold estimation. This study also discussed the frequency specificity, and detection considerations with both evoked potentials (Cone-Wesson et al., 2002). These findings were supported by a separate study by Vender Werff and colleagues. Their group studied a group of 32 children and found strong correlations between ASSR and click ABR thresholds. They suggested that the ASSR is a reasonable alternative for predicting auditory thresholds in children (Vander Werff et al., 2002).

While both techniques could be used to predict pure-tone thresholds, a key comparison to be made between the ABR and the ASSR is that even though the ASSR bypasses the subjective nature of detecting the response akin to the ABR, it does not produce waveforms that are analysed via their morphology. As aforementioned, the analysis of the ABR waveform is a key feature for otoneurologic applications and differential diagnosis

providing valuable information to the clinician about potential sites of lesion. While the efficient automatic detection algorithms of the ASSR allow clinicians to quickly and accurately estimate hearing thresholds, diagnostic information is lost as the generation of the ASSR requires the individual waveforms of the response to overlap when the frequency modulation is applied at 40Hz. The detection of the ASSR relies on using a fast Fourier transform of the responses, which can be done automatically rather than by the close visual analysis of the original wave response. This thesis is aimed to achieve the time-saving characteristics of applying multiple stimuli simultaneously like the ASSR but retain the advantages of having a waveform that can be used for differential diagnosis.

1.15 Interleaved Stimuli

It is an objective for researchers and clinicians to minimize the ABR test time without maximising the stimulus rates to levels which could compromise the waveforms. The ASSR has been a well-established method for recording responses with multiple stimuli that activate different populations of neurons simultaneously. Because the repetition rate of each stimulus may be different, not only do different parts of the neural system get activated but there may also be a time difference between each stimulus. Thus, it is assumed that each recording captures the activity of different sets of frequency specific neurons and multiple recordings can be obtained at the same time (Cebulla, Sturzebecher, Don & Müller-Mazzotta, 2012).

The stimulus paradigm in this study is designed to reap the same time-saving advantages of applying multiple stimuli to produce ABR recordings in a shorter test time. However, the proposed stimulus used is pseudo simultaneous, such that there is a slight time difference between each click rather than being completely simultaneous. Experiments involving multiple stimulus conditions may employ a block or interleaved paradigm. Block paradigms involve presenting each stimulus type separately (e.g. Block 1: A A A, Block 2: B B B). In an interleaved paradigm the different stimulus types are presented alternately (e.g. A

B A B) (Skoe & Kraus, 2010). An interleaved paradigm will be used in this experiment and this design is critical because theoretically, the neuronal pathway evoked by stimulus "A" has time to recover as stimulus "B" activates a separate neuronal pathway and conversely, the neuronal pathway activated by stimulus "B" has time to recover while stimulus "A" is presented. This interleaved presentation pattern could potentially minimise the effects of neural fatigue and adaptation, because each part of the neural system responding to one type of stimulus has time to recover as another part of the neural system is being stimulated by a different type of stimulus. The stimuli are staggered such that they are not evoking a response at the exact same time but rather, they are presented in an alternating pattern. This creates a temporal difference between each stimulus that may produce robust recordings of different parts of the cochlea. These types of stimuli shall be termed "interleaved stimuli" in this thesis.

Currently, most ABR recordings performed clinically deliver thousands of one type of stimuli sequentially to one ear at a time and once one set of recording is complete, the clinician adjusts the parameters of the next stimulus or chooses to present to the other ear. The custom-written software used in this project (Te Pihareinga; O'Beirne, 2015) is capable of rapidly interleaving stimuli at different frequencies and intensities and is able to use different transducers into different ears. Broadband click stimuli were used for this project as these stimuli activate a larger area of the BM. Technical issues during the project prevented the use of chirp stimuli, but nevertheless click stimuli are an excellent tool used in ABR recordings to quickly capture the general function of the cochlea at different intensities. The used paradigm involved interleaving click stimuli presented to one ear and then the other, meaning the interleaved stimuli should be separated both temporally and in the site of activation. This paradigm was designed to rapidly interleave stimuli to be nearly

simultaneous, enabling the observer to capture multiple recordings in the almost the same time it would take to conduct one measurement.

Clinically, interleaved click stimuli delivered to both ears are used to measure AEPs during intraoperative monitoring. This method is effective because by simultaneously recording both ears it reduces the time where one ear is not being monitored and provides more rapid feedback to the surgeons (Galloway, Nuwer, Lopez & Zamel, 2010). Another clinical application of interleaved stimuli is with some automated ABR systems (Mason, 2012). There is relatively recent and growing body of research utilizing interleaved stimulation to evoke the ABR. Although, these studies often utilize trains of frequency specific stimuli presented to the same ear, interleaving stimuli with different frequencies or rate rather than interleaving between the ears. An example of this is chained stimuli such as the Gliding HIghpass NOise MAsker (GHINOMA) used by Petoe and colleagues (2009). The chain of tone-burst stimuli they used consisted of 8 different frequencies between 500 Hz to 6 kHz. By presenting the frequencies in a descending order, forward masking was thought to be the reason for the improvement in low frequency ABR using the GHINOMA stimuli and overall this stimulus was shown to reduce test-time. (Petoe, Bradley & Wilson, 2009). Buran and colleagues (2019) proposed that by selecting and ordering interleaved tone stimuli frequencies and levels to the same ear effectively, ABR acquisition time may be reduced while minimising the effects of adaptation. Buran's group developed and validated an interleaved stimulus paradigm that reduced ABR acquisition time with minimal effects on the ABR threshold and latency using an open-source data acquisition software. It has been noted that the lack of adoption of interleaved stimuli paradigms have been attributed to the limited availability of suitable software (Buran et al., 2019).

Cebulla and colleagues (2012) conducted a study on 11 otologically healthy adults utilizing interleaved broadband chirp stimuli with different repetition rates (20/s and 22/s) in

the same ear. Their results showed patterns resembling the effects of forward masking with the wave V amplitude decreasing and latency delay being present. This paper focused on the recording interactions and possibility of assessing temporal aspects of peripheral auditory processing. The study proved the possibility of using interleaved trains of broadband chirps presented at different presentation rate producing reliable ABRs in normal hearing adults (Cebulla, Sturzebecher, Don & Müller-Mazzotta, 2012). A different study investigated the effects of presenting a sequence of 20 tone-bursts at four different frequencies and five intensities (to the same ear) on the ABR response in mice. No differences in thresholds, latencies or amplitudes of the responses were observed for these 20 tone-burst sequences compared to when the stimuli are presented individually. These results suggest that minimal adaptation effects are exhibited at the cochlear and brainstem level using a tone-burst sequence at different frequencies and intensities. Furthermore, significant time-savings were achieved compared to presenting the different types of tone-bursts sequentially (Mitchell, Kempton, Creedon & Trune, 1996).

A recent study by Polenka and Maddox (2019) described a new method called the parallel ABR (pABR) which presents randomly timed tone-bursts at 5 different frequencies to both ears simultaneously. Similar to the present study, their presentation paradigm was designed to overcome the test duration problems of current acquisition methods which evoke responses using periodic sequences of tone-bursts sequentially to one ear at a time. However, the pABR design elicits the ABR from both ears simultaneously compared to the interleaved fashion the present study utilizes and they used frequency specific tone-bursts instead of clicks. They were able to demonstrate that high-quality waveforms at multiple frequencies could be produced using the pABR technique in 10 normal hearing adults while reducing overall test time.

There have been interleaved types of stimuli researched that are aimed to evoke multiple AEPs at the same time, for example, eliciting the brainstem and cortical brain potentials simultaneously. These studies have been limited by both technological and physiological barriers. Bidelman and colleagues (2015) designed an interleaved stimulation technique to acquire the brainstem following response and the auditory late response in a single measurement by presenting a series of high then low presentation rates alternately. This method was found to be a faster protocol and offer 3 times increase in recording efficiency compared to standard presentation methods (Bidelman, Weiss, Moreni & Alain 2015). This presentation paradigm was adapted by Kohl et al. (2018) interleaving high rate deconvolution sequences and single low rate presentations (Kohl et al., 2018) to simultaneously and effectively acquire the ABR, AMLR and ALR in 20 normal hearing participants. The results suggest that their proposed interleaved stimulus presentation is a promising toolset for fast and reliable simultaneous acquisition of full-range auditory evoked potentials.

The main distinguishing feature between previous research on interleaved stimuli and this experiment is that most previous studies have interleaved stimuli of different frequency, rate and intensity levels to the same ear. In contrast this experiment will interleave the same type of stimuli between the ears. Other studies that evoke ABRs in both ears generally evoke the ABR simultaneously rather than interleaving the stimuli. Although clinically, interleaved stimuli between the ears are used in intraoperative monitoring and automated ABR, the stimuli paradigm in this experiment is designed so that visual ABR waveforms are still produced, thus providing more diagnostic information. Not only does this proposed paradigm indicate the presence or absence of a waveform, but it could also potentially yield clear waveforms that could provide supplementary information to clinicians.

1.16 Central vs Peripheral Effects

When two stimuli are interleaved between the left ear (L) and the right ear (R), then the neuronal pathway activated by stimulus "L" has time to recover while stimulus "R" is being presented and vice versa. For example, an interleaved stimulus presented to both ears where the overall click rate is 90.9 clicks/s and an overall ISI of 11 ms means that either the left ear or right ear is being stimulated at a rate of 90.9 times/s. In other words, the central nervous system receiving signals from both ears are being stimulated at a "central" rate of 90.9/s. However, each ear (the peripheral system) is only being stimulated 45.45 times/s, essentially stimulated at half of the overall click rate. While the "central" click rate is 90.9 clicks/s, the "peripheral" click rate at which one ear is being stimulated is only 45.45 times/s. The "peripheral" ISI and therefore time for recovery that the peripheral auditory system that one side has is 24 ms even though the ISI of the central nervous system is only 12 ms. This is the theoretical basis for the minimal adaptation effects hypothesized in this interleaved paradigm under the *main assumption that stimuli presented to either ear stimulate different populations of neurons.*

This is true for either the left and right peripheral auditory pathway (i.e. the cochlea and peripheral auditory nerve), as these pathways are separate initially. However, we know that to some extent, the central auditory pathway (i.e. the brainstem), merges signals coming from both the peripheral auditory systems. As explained in chapter 1.5, certain neural generators of the ABR process and integrate information from both ears. Potentially interleaved stimuli are separate as they are processed in the peripheral auditory system then have some form of interaction as they travel up the central auditory system in the brainstem. A heavily studied area of research regarding the interaction of simultaneous stimuli is the binaural interaction component (BIC). The BIC occurs when the sum of the monaural responses of the AEP does not equal the binaural response. As the name suggests, it is an

interaction of the activity along the auditory pathway when stimuli are presented binaurally supporting the hypothesis that there is interaction of auditory signals centrally when two simultaneous signals are presented to both ears at the same time. The BIC contributes to about 14-23% of the expected amplitude of binaural AEPs. The BIC is often denoted as a β-wave is derived by subtracting the ABR recording by binaural clicks from ABR recording from monaural clicks (Brantberg, Fransson, Hansson & Rosenhall, 1999, Levine, 1981; Tolnai & Klump, 2018). It should be noted that the BIC has been demonstrated for binaural stimuli presented simultaneously and is unlikely to be present in responses to interleaved stimuli with larger inter-stimulus intervals.

In contrast, there are studies that provide strong evidence for the hypothesis that the signals coming from the left and right ear remain separate even centrally. While some studies have attributed adaptation effects to the cochlear nerve and central brainstem regions, some researchers have also considered that the adaptation effect is peripheral and potentially localized at the junction between the cochlear nerve and hair cells (Eggermont & Odenthal, 1974). Don, Allen and Starr (1977) argued that although wave V corresponds to activity originating from the midbrain, observed latency shifts indicating adaptation can be attributed to changes occurring peripherally. They conducted an experiment showing wave V latency shifts observed due to high click rates are exclusively attributable to inputs from the stimulated ear and minimally affected by contralateral stimulation which suggests minimal central adaptation effects.

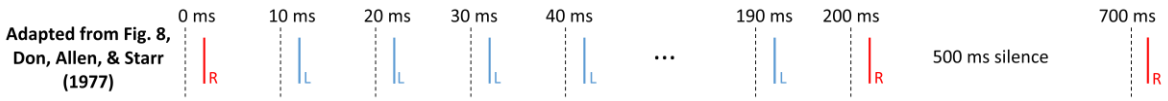

Figure 13. The schematic diagram adapted from Figure 8 of Don et al. (1977). A train of 20 clicks at 40 dB was presented at 100/s with the first click presented to the right ear acting as the control signal. The following 18 clicks are presented to the left ear. The 20th click of the train is again presented to the right ear. There is a 500 ms silence before the start of the next train of 20 clicks.

Don and colleagues (1977) proposed that if the adaptation was a binaural or central process then the 18 adapting stimuli presented to the left ear would cause a wave V latency shift in the 20th click in comparison to the first control click. Their results showed that the 20th click was not significantly different to the first click and furthermore, the 18th or last click presented to the left ear showed reduced wave V amplitude and increased latency. Therefore, they concluded that adaptation, marked as a shift in wave V latency is confined to the pathway of the stimulated ear and is not significantly altered by stimuli presented from the contralateral ear (Don, Allen & Starr, 1977). With this information, this study proceeded with the assumption that adaptation of the ascending auditory pathway is confined peripherally and that interleaved stimuli alternating between the ears activate separate populations of neurons. Therefore, a stimulus presented to one ear will only be affected (in terms of adaptation) by subsequent stimuli presented to the same ear and not by stimuli presented to the contralateral ear.

1.17 Statement of Purpose

Performing ABR in a shorter amount of time offers many advantages and therefore, the research question of this project was to find out if the rapid interleaving of ABR stimuli with different characteristics and delivery modes offer practical advantages in terms of

response quality (i.e. SNR and waveform morphology) and test time, without compromising diagnostic accuracy. The study focused on the wave V latency and amplitude measurements as an indication of adaptation, while the Fsp value was used to indicate the quality of the waveforms.

Four main recording conditions were recorded for each participant: two from the binaural interleaved recordings from the left and right ears called “Interleaved R” and “Interleaved L”. “Monaural fast” is used here to refer to the recording condition that is presented to the right ear at the rate equivalent to the overall combined rate of the interleaved recordings. “Monaural slow” is the fourth recording condition and refers to monaural stimuli presented to the right ear at the rate at which only the “Interleaved R” is being presented, essentially half the rate of the “Monaural fast”. These four recording conditions are what were compared statistically and were measured at three different rate combinations: 11/22 ms ISI (90.9/s, 45.5/s), 13/26 ms ISI (76.9/s, 38.5/s) and 15/30 ms ISI (66.7/s, 33.3/s). It was hypothesized that:

1. The wave V latency measures for “Monaural Fast” traces are significantly different than the wave V latency measures for the “Monaural Slow” at each of the three rate combinations.
2. The wave V latency measures for “Monaural Fast” traces are significantly different from the wave V latency measures for the “Interleaved R” at each of the three rate combinations.
3. The wave V latency measures for “Interleaved R” traces are not significantly different than the wave V latency measures for the “Monaural Slow” at each of the three rate combinations.

4. The wave V amplitude measures for “Monaural Fast” traces are significantly different than the wave V amplitude measures for the “Monaural Slow” at each of the three rate combinations.
5. The wave V amplitude measures for “Monaural Fast” traces are significantly different from the wave V amplitude measures for the “Interleaved R” at each of the three rate combinations.
6. The wave V amplitude measures for “Interleaved R” traces are not significantly different than the wave V amplitude measures for the “Monaural Slow” at each of the three rate combinations.
7. The number of sweeps required for the “Monaural Fast” traces to reach an Fsp of 3.1 are significantly different than the number of sweeps required for the “Monaural Slow” traces to reach an Fsp of 3.1 at each of the three rate combinations.
8. The number of sweeps required for the “Interleaved R” traces to reach an Fsp of 3.1 are significantly different than the number of sweeps required for the “Monaural Fast” traces to reach an Fsp of 3.1 at each of the three rate combinations.
9. The number of sweeps required for the “Interleaved R” traces to reach an Fsp of 3.1 are not significantly different than the number of sweeps required for the “Monaural Slow” traces to reach an Fsp of 3.1 at each of the three rate combinations.

In other words, it is hypothesized that the wave V latency for the “Monaural Fast” traces are significantly longer and wave V amplitude to be significantly smaller than both the wave V latencies and wave V amplitude of the “Monaural Slow” and “Interleaved Right” traces. It is also expected that it takes a significantly higher number of sweeps for the “Monaural Fast” traces to reach an Fsp of 3.1 compared to the “Interleaved R” and

"Monaural Slow" traces. Meanwhile, there should be no significant differences in the wave V amplitude, latency and Fsp results between "Interleaved R" and "Monaural Slow" traces.

If these hypotheses are proven correct, then this suggests that interleaved stimuli are able to yield results at the rate of the "Monaural Fast" recordings but not exhibit adaptation effects observed at higher stimulus rates (i.e. reduced amplitude and increased peak latencies). Furthermore, it means that interleaved stimuli could still produce waveforms with similar characteristics in terms of amplitude and latency as presenting a monaural stimulus at half the presentation rate.

Chapter 2: Methods

2.1 Calibration of the E-A-RTONE insert earphones

The peak voltage drive to the E-A-RTONE ABR insert earphones was adjusted in software to be identical to that provided by an Interacoustics Eclipse that had recently been calibrated with those same transducers. The Eclipse was calibrated in dB nHL for short duration signals by ECS Ltd, using calibration figures provided to them by the University of Auckland. This calibration was valid for tone-bursts at 500 Hz, 1 kHz, 2 kHz, and 4 kHz, and for clicks. Only clicks were used in this study. Shown in Figures 14 and 15 is evidence that the voltage drive to the E-A-RTONE ABR transducer is the same as the Eclipse at 60 dB nHL and linear between 20 and 90 dB nHL.

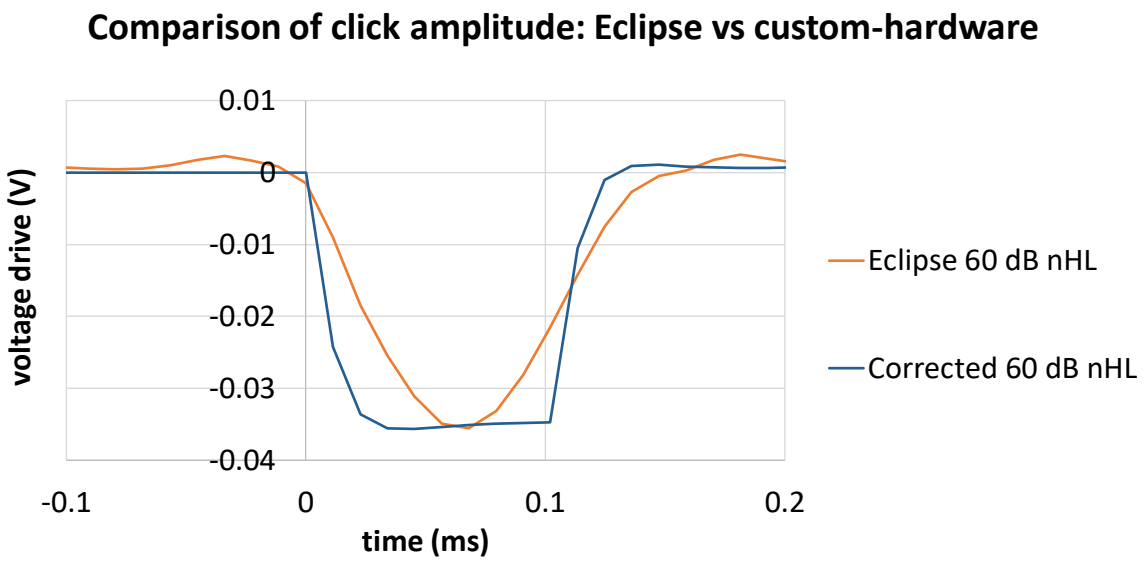

Figure 14. Comparison of voltage drive for a 60 dB nHL click from the Interacoustics Eclipse and from our custom hardware after calibration corrections. Both outputs were recorded driving into the E-A-RTONE ABR transducer.

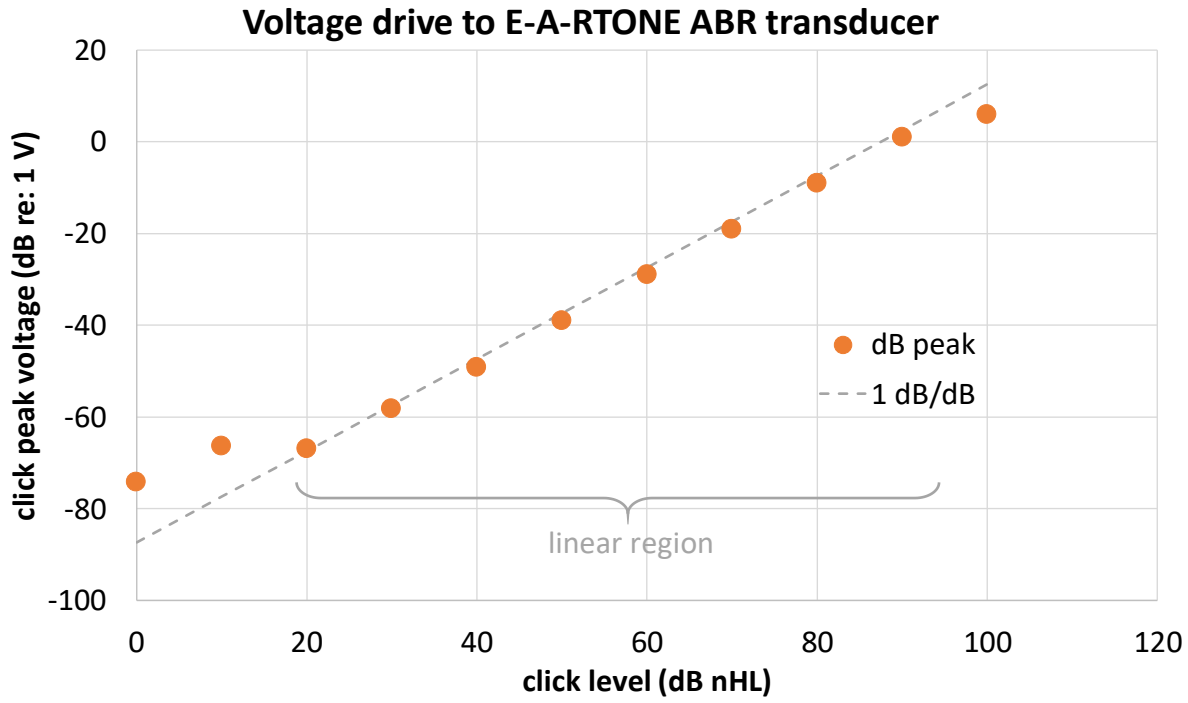

Figure 15. The voltage drive recorded from our custom evoked potential system to the E-A-RTONE ABR transducer is linear for clicks between 20 and 90 dB nHL.

2.2 Materials and Equipment

Pure-tone audiometry testing were conducted in soundproof testing booths at the University of Canterbury Audiology Clinic meeting the standard of the International Organization for Standardization [ISO] 8253-1:2010, using either calibrated GSI Audiostar Pro or GSI 61 diagnostic audiometers with 3M E-A-RTONE GOLD 3A insert earphones.

ABR recordings were measured and processed using custom software designed by Professor Greg O'Beirne at the University of Canterbury called Te Pihareinga (O'Beirne, 2015). This software was run on an HP Revolve 810 laptop, connected to a NI cDAQ-9174 data acquisition system (National Instruments, TX, USA): The system's NI 9269 module produced the audio stimuli, which were then amplified by a Rolls Stereo Mini-mix VI sound amplifier before being passed to the E-A-RTONE ABR insert earphones, while the NI 9222

module recorded the output from the CED1902 Mk III biological amplifier (Cambridge Electronic Design Ltd., Cambridge, UK) to which the participants were connected.

A vertical montage was used for the ABR recordings as shown in Figure 6, with the active electrode placed on the high forehead, the indifferent electrode on the nape of the neck at the midline, and the earth electrode placed on the clavicle. Ambu BlueSensor N electrodes (Ambu A/S, Ballerup, Denmark) were used for all recordings.

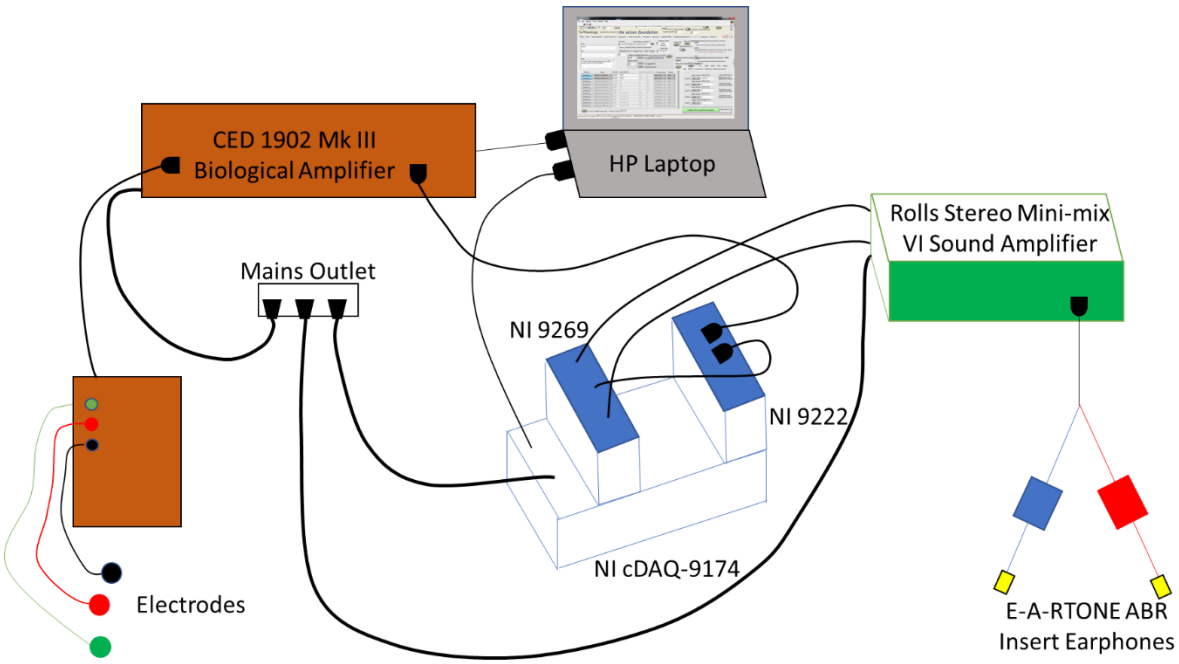

Figure 16. Schematic diagram of the ABR acquisition system.

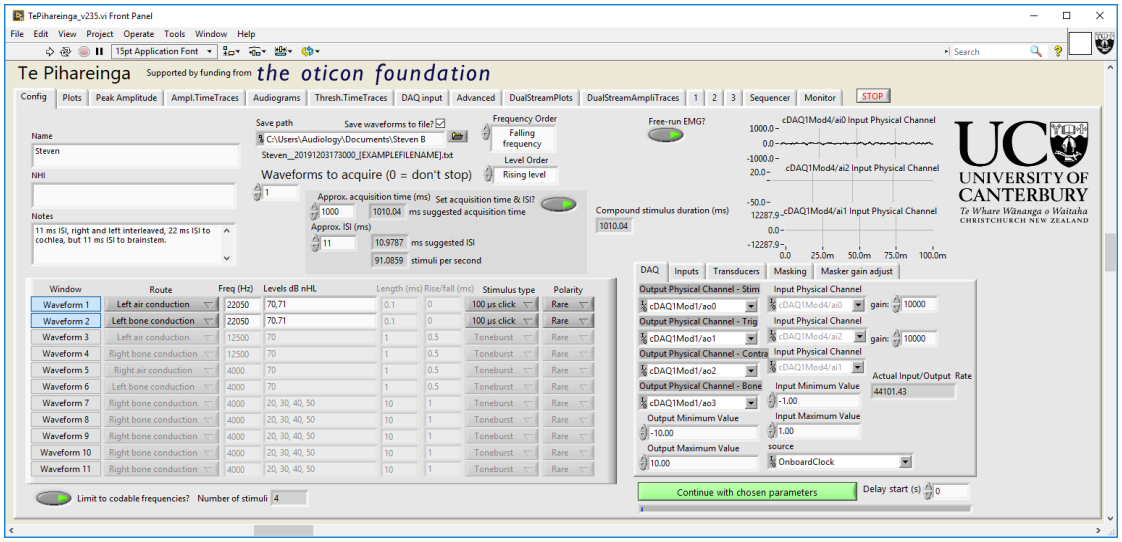

Figure 17. Screenshot of the Te Pihareinga software showing the main configuration page. Numerous setting specifications such as the number of recordings, number of channels, route, stimulus frequency and levels can be configured on this page. A free-running EMG and electrical noise plot are shown at the top right of the screen.

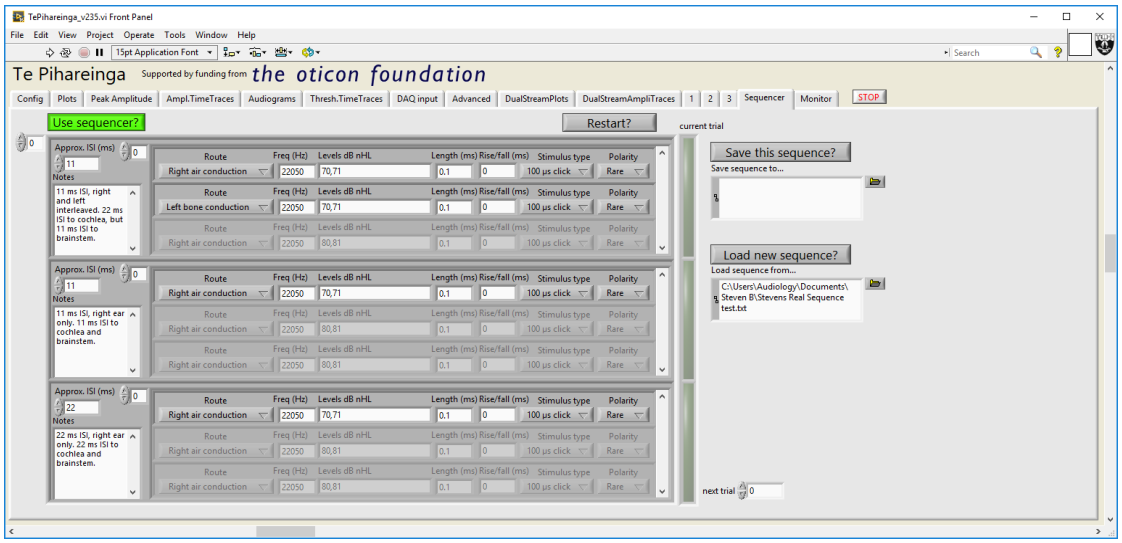

Figure 18. Screenshot of the Te Pihareinga software showing the sequencer feature. The recording parameters of the first three recordings are shown above. The first recording is interleaved with two routes being activated, one presenting clicks to the right ear and one to the left ear. The “Left bone conduction” channel is in fact air conduction but is labelled differently.

2.3 Participants

The subjects for the initial trialling and troubleshooting phase of the study were the primary investigator and his primary supervisor. Once the system was ready, recruitment of the main participant pool followed.

The participants in the main experiment consisted primarily of staff and students from the University of Canterbury and were recruited via word of mouth and advertisement posters (See Appendix D). Additional participants were recruited via word of mouth from outside the university. The criteria for inclusion in the study were that participants must be above 18 years old and have normal pure-tone thresholds (≤ 15 dB HL between 250 Hz - 8 kHz bilaterally).

All participants completed air conduction pure-tone audiometry testing from 250 Hz-8 kHz prior to the ABR testing (see Table 1 for a summary of their age, sex and audiometric thresholds). Participants 4, 15 and 21 had high-frequency thresholds outside of the normal range – while they also proceeded to the main part of the study, their results were excluded in the analysis. Participant 10 produced invalid results due to equipment error and was also excluded in the analysis. All participants included in data analysis had normal hearing, no significant otologic history and normal outer ear health through otoscopic examination. There were 19 participants included in data analysis (4 males and 15 females) with the participant's ages ranging from 21 to 39 years old (M = 26, SD = 4.52).

Table 1.

Participant Data and Audiometric Thresholds.

Participant			Audiometric AC Thresholds (dB HL)															
#	Age	Sex	R Frequencies (kHz)								L Frequencies (kHz)							
			0.25	0.5	1	2	3	4	6	8	0.25	0.5	1	2	3	4	6	8
1	25	F	-5	0	0	5	5	0	5	5	0	0	0	0	0	0	5	0
2	24	M	10	0	0	-5	-5	-10	-10	-10	0	0	0	-5	-5	-10	-5	-10
3	23	F	-5	-5	5	-5	-5	5	5	5	-5	-5	5	5	5	0	5	0
4*	40	F	15	10	10	5	10	20	20	25	15	15	10	15	10	25	25	20
5	23	F	5	-5	-5	5	0	0	5	0	0	-5	0	-5	-5	-5	-5	5
6	22	F	0	0	-5	-5	-5	-5	0	-5	5	0	0	0	-5	0	0	-5
7	24	F	-5	-5	5	5	0	0	0	10	-5	0	0	10	5	-10	15	5
8	24	F	-5	-5	-5	0	10	0	0	5	0	-5	5	-5	5	-5	0	0
9	39	F	5	10	5	0	5	5	5	5	5	10	5	5	5	5	0	5
10*	28	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	21	M	0	-5	-5	-5	-5	-5	0	0	0	-5	0	-5	-5	-5	0	5
12	28	F	5	5	5	0	5	5	5	15	5	5	5	10	5	5	0	0
13	31	F	5	5	5	5	5	0	5	0	5	10	5	5	0	0	15	0
14	33	F	10	5	5	5	0	0	0	-5	0	0	5	5	5	0	5	0
15*	57	F	15	15	15	15	15	15	15	25	15	15	15	10	15	10	15	30
16	27	F	0	0	0	10	15	15	10	-5	5	0	0	5	15	10	5	5
17	25	F	-5	10	-5	0	0	-5	0	0	0	10	0	5	0	-5	15	0
18	25	F	5	5	5	10	10	0	5	0	5	5	5	5	5	5	5	5
19	26	M	5	0	0	5	5	0	0	0	10	5	5	0	0	0	0	0
20	30	F	5	5	15	10	10	5	10	10	5	5	10	5	5	0	5	5
21*	30	F	0	10	10	10	5	0	5	20	15	15	20	5	5	5	10	15
22	24	F	0	0	5	5	10	5	5	-5	0	5	5	10	10	10	-10	5
23	21	M	-5	0	5	5	5	5	-5	-5	0	0	5	5	5	-10	0	0

Note. Participants who were excluded in data analysis are marked by an asterisk.

Table 1 shows the air conduction audiometric thresholds for all 23 participants who had pure-tone audiometry testing. Only 19 normal hearing participants were included in the data analysis.

2.4 Procedure

Ethical approval for this study was granted by the University of Canterbury Human Ethics Committee (HEC Application 2019/17LR; See Appendix A). Otoscopy and pure-tone

audiometry were performed on each participant following the Audiology Clinical Protocol and Guidelines of the University of Canterbury. This was to check that each participant had normal hearing and a healthy outer and middle ear to be suitable to proceed with audiometry and ABR testing. Insert earphones were used to measure air conduction thresholds at the standard frequency range 250 Hz - 8 kHz.

If normal hearing thresholds are determined, the participant was invited to participate in the main part of the study. ABR testing was conducted in a quiet room and took place in one session between 1 to 1.5 hours. The participants were instructed on what to expect for the testing and were advised to remain as relaxed as possible while seated on a reclined couch. The electrode sites on each participant's head as described in Figure 6 were prepared by wiping the site with alcohol wipes and these areas were lightly exfoliated using 3M Red Dot Trace Skin Prep tape to reduce the impedance of the electrode connection. BlueSensor ECG electrodes were then placed on the placement sites and connected to the corresponding electrode wires. The participants were fitted with 3M E-A-RTONE ABR insert earphones through which the click stimuli were presented.

To reduce the need for tester intervention during the recordings, and to save time between them, the custom-written evoked potential software was designed to be capable of carrying out a pre-programmed sequence of ABR recordings using different settings. The settings that could be changed between recordings included stimulus parameters (types, frequencies, intensities, durations, rise/fall time, polarity, routes), the interstimulus interval, and the comments that were saved with the data.

Table 2.

Recording Parameters for the Nine Sets of ABR Recordings Measured for Each Participant

Setting	Presentation Ear	Central ISI	Peripheral ISI	Click type	Stimulus level (dB nHL)
1	Interleaved binaural	11 ms (90.91/s)	22 ms (45.45/s)	100 µs rarefaction	70, 71 dB nHL
2	Monaural right ear	11 ms (90.91/s)	11 ms (90.91/s)	100 µs rarefaction	70, 71 dB nHL
3	Monaural right ear	22 ms (45.45/s)	22 ms (45.45/s)	100 µs rarefaction	70, 71 dB nHL
4	Interleaved binaural	13 ms (76.92/s)	26 ms (38.46/s)	100 µs rarefaction	70, 71 dB nHL
5	Monaural right ear	13 ms (76.92/s)	13 ms (76.92/s)	100 µs rarefaction	70, 71 dB nHL
6	Monaural right ear	26 ms (38.46/s)	26 ms (38.46/s)	100 µs rarefaction	70, 71 dB nHL
7	Interleaved binaural	15 ms (66.67/s)	30 ms (33.33/s)	100 µs rarefaction	70, 71 dB nHL
8	Monaural right ear	15 ms (66.67/s)	15 ms (66.67/s)	100 µs rarefaction	70, 71 dB nHL
9	Monaural right ear	30 ms (33.33/s)	30 ms (33.33/s)	100 µs rarefaction	70, 71 dB nHL

The sequence of recording parameters for this experiment is shown in Table 2. The first setting programmed was an interleaved binaural click stimulus between the left and right ears at an overall rate (or central rate) of 90.91clicks/s. Once the recordings from each setting finished, the next recording setting was applied after a five second pause. The second setting in this sequence used monaural click stimuli presented to the right ear with an ISI of 11 ms and rate of 90.91 clicks/s. The third setting presented monaural click stimuli to the right ear at an ISI of 22 ms and therefore rate of 45.45 click/s. This sequence of three recordings was then repeated but with an ISI combination of 13/26 ms, and 15/30 ms. For all nine recordings, two replicate traces were measured. In practice, this was achieved by using stimuli presented one decibel apart (a difference so small as to be clinically insignificant), such as 70 and 71 dBnHL. For clarity, both waveforms are presented as belonging to the

lower of these two levels (i.e. traces shown as 70 dB nHL in figures were recorded at either 70 or 71 dB nHL).

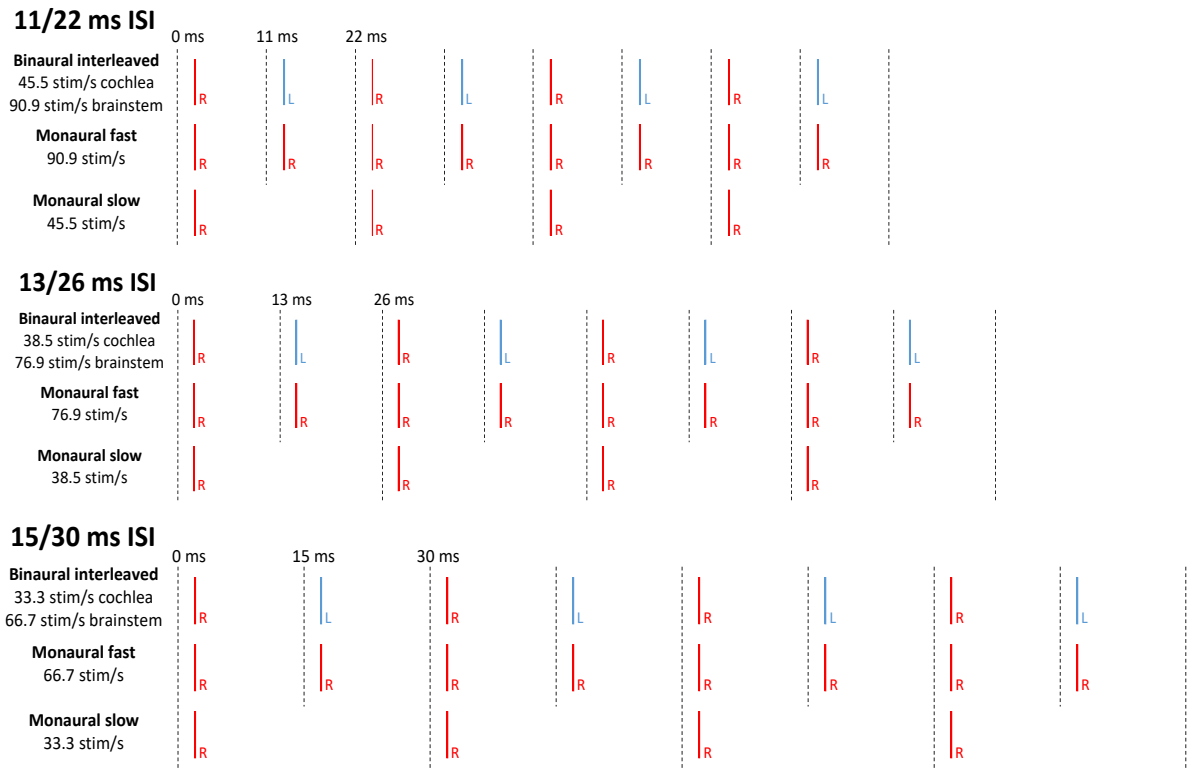

Figure 19. Schematic diagram illustration of the nine click conditions at three sets of interstimulus intervals (x-axis drawn to scale).

In Figure 19, time is represented along the X axis. Starting on the point marked 0 ms, each coloured bar represents the point in time in which a click is presented to either the right ear (R; red) or left ear (L; blue) in regular intervals according to the rate at which the stimuli are presented. The dotted lines prior to each stimulus indicates the start of each new recording window, therefore the time between each dotted line and stimulus is the 2 ms pre-stimulus period. For the three different sets of interstimulus intervals, the first recording is the interleaved setting at which each stimulus (either to the left or right ear) is presented at an overall or "central" interstimulus period, but each subsequent stimulus that is coming from

the same ear is presented at twice the interstimulus interval. The second stimulus condition labelled “Monaural fast” is monaural clicks presented to only to the right ear, at the overall or “central” rate of the previous binaural stimulation. The “Monaural slow” condition is another monaural click condition presented to only the right ear but at the rate at which only one of the ears is stimulated (“peripheral” rate) in the binaural condition.

For the interleaved setting, either of the ears are stimulated together at a central rate of 76.9 clicks/s (13 ms ISI centrally). Potentially, the central brainstem regions have a recovery period (13 ms) determined by signals coming from both ears. The stimulation rate that one of the ears are stimulated to is essentially halved at a “peripheral” rate (38.46 clicks/s) and has double the recovery period (26 ms) compared to the central brainstem pathways.

The stopping criteria for each recording is a maximum of 3000 averages. This number of averages was selected as it is a large enough to potentially produce high SNR but not too many to require an excessively long period of time for each trace to finish. During each recording and for both replicates, the Fsp value was continually measured. On the software, a horizontal line on the Fsp graph is present to mark when each recording reaches a Fsp value of 3.1, indicating that it has reached an optimal signal to noise ratio for it to be a viable and clear trace. The rejection criteria at the front end of the recordings is 20- 30 µV peak.

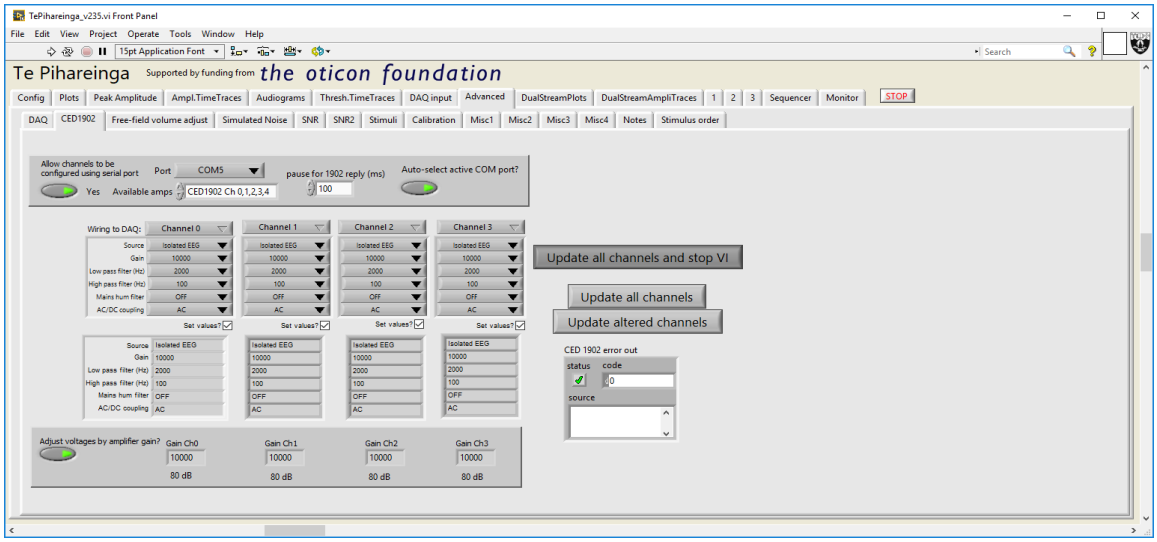

Figure 20. Screenshot of the Te Pihareinga software showing the DAQ settings. The CED 1902 amplifiers are programmable via serial port using RS232 protocol. Each channel is AC coupled and set to 10000x gain, with a low pass filter of 2 kHz, and a high pass filter of 100 Hz.

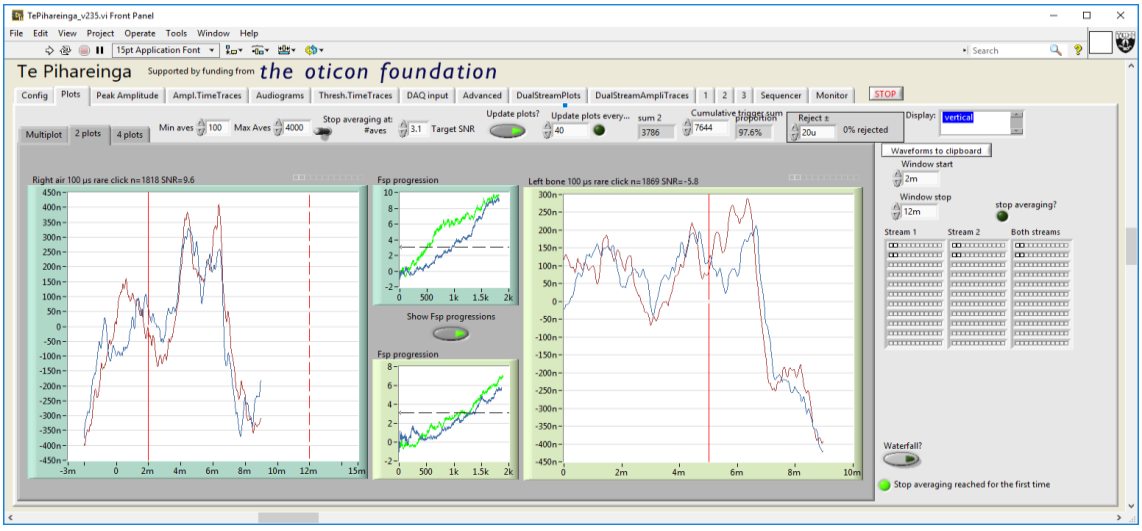

Figure 21. Screenshot of the Te Pihareinga software showing an example of a live recording plot. The top of the page shows the option to set the minimum and maximum averages required, target SNR. The cumulative number of clicks presented, and percentage of recordings rejected are also shown. In this example, an interleaved recording is being

performed with a live average of the recording being shown. A live Fsp progression plot is of both the left and right air conduction recordings are shown in the middle.

Chapter 3: Results

Once all ABR recordings for each participant were completed, a template was created on Microsoft Excel to transfer all data and graph all the results. The results recorded from Te Pihareinga were comprehensive and included substantial metadata alongside the waveforms. Analysis of the waveforms were performed by the primary investigator and checked by the primary supervisor. The wave III and V peaks were identified by visual inspection. The Excel template was designed to automatically calculate and record the peak amplitudes and latency of each waveforms upon manually marking the boundaries of the wave III and wave V peaks.

The study design allows for analysis via a series of simple repeated measures ANOVA for each ABR measure. The hypotheses were tested at the three sets of rates separately. The nine sets of recordings (shown in Table 2) were carried out for each of the 19 participants. Descriptive statistics of wave V amplitude and latency and number of sweeps were calculated for each stimulus conditions separately. A series of nine repeated measures ANOVA were conducted to determine if there were any significant differences in the wave V amplitude, wave V latency and number of sweeps required to reach an Fsp of 3.1 in the "Interleaved R", "Monaural Fast" and "Monaural Slow" recording conditions at each rate combinations. Although there was a disproportionate number of male and female participants (4 males and 15 females), gender effects were not being tested and each test condition group that was being compared consisted of the same 19 participants. A p value < 0.05 was used to determine statistical significance. All statistical analysis was conducted by the primary investigator and checked by the primary and secondary supervisors. In this section, analysis of the Wave V latency will be presented first followed by analysis of Wave V amplitude then number of sweeps to Fsp of 3.1.

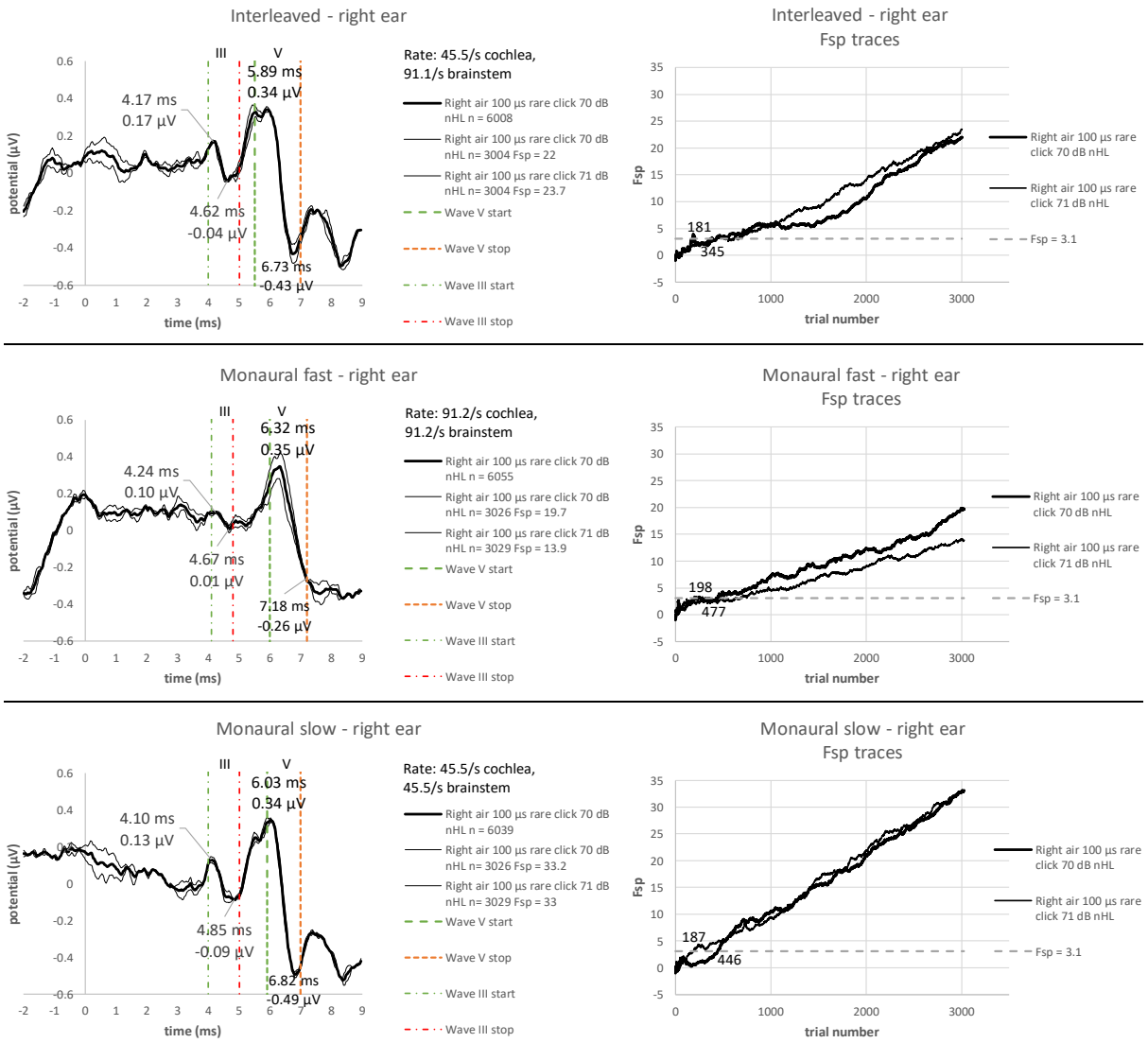

Figure 22. Left column: Sample ABR waveforms from Participant 16 highlighting wave V latency differences for the Interleaved R, Monaural Fast and Monaural Slow recording conditions at the 11/22 ms ISI rate combination. Right column: Fsp traces for these ABR recordings, with labels showing the trial number at which the trace first exceeded Fsp = 3.1.

3.1 Wave V Latency

The wave V latency values (ms) recorded and measured for each participant at all stimulus conditions are shown in Table 3, Table 4 and Table 5. These latency values are the latency values of the two replicates of 3000 traces added together.

Table 3.

Participant Wave V Latency (ms) Data for the 11/22 ms ISI Recordings

	11/22 ms ISI			
	Interleaved R	Interleaved L	Monaural Fast	Monaural Slow
Participant 1	6.57	6.50	7.05	6.53
Participant 2	6.57	6.37	6.57	6.57
Participant 3	6.50	6.21	6.82	6.53
Participant 5	6.07	6.19	6.25	6.03
Participant 6	6.34	6.44	6.78	6.44
Participant 7	5.85	6.00	6.39	5.87
Participant 8	6.09	6.03	6.34	6.03
Participant 9	6.50	6.48	6.68	6.44
Participant 11	6.41	6.64	6.50	6.25
Participant 12	6.30	6.09	6.46	6.19
Participant 13	6.21	6.23	6.48	6.07
Participant 14	6.55	6.66	6.75	6.71
Participant 16	5.89	6.05	6.32	6.03
Participant 17	6.05	6.09	6.55	6.09
Participant 18	6.50	6.44	6.71	6.41
Participant 19	6.62	6.46	6.73	6.62
Participant 20	6.03	6.03	6.32	5.91
Participant 22	6.48	6.32	6.78	6.50
Participant 23	6.62	6.62	6.84	6.53

Table 4.

Participant Wave V Latency (ms) Data for the 13/26 ms ISI Recordings

	13/26 ms ISI			
	Interleaved R	Interleaved L	Monaural Fast	Monaural Slow
Participant 1	6.75	6.37	6.93	6.55
Participant 2	6.53	6.41	6.87	6.48
Participant 3	6.50	6.57	6.66	6.64
Participant 5	6.00	6.14	6.21	6.00
Participant 6	6.37	6.46	6.80	6.44
Participant 7	6.00	6.00	6.07	6.00
Participant 8	6.03	5.91	6.30	6.00
Participant 9	6.37	6.09	6.57	6.30
Participant 11	6.50	6.55	6.68	6.44
Participant 12	6.23	6.07	6.44	6.19
Participant 13	6.05	6.25	6.37	6.12
Participant 14	6.84	6.59	6.73	6.91
Participant 16	6.00	5.94	6.23	5.91
Participant 17	6.12	6.19	6.39	6.14
Participant 18	6.23	6.37	6.62	6.41
Participant 19	6.50	6.44	6.84	6.44
Participant 20	5.91	5.96	6.32	5.94
Participant 22	6.44	6.37	6.73	6.41
Participant 23	6.55	6.53	6.66	6.66

Table 5.

Participant Wave V latency (ms) Data for the 15/30 ms ISI Recordings

	15/30 ms ISI			
	Interleaved R	Interleaved L	Monaural Fast	Monaural Slow
Participant 1	6.53	6.44	6.87	6.44
Participant 2	6.55	6.34	6.78	6.50
Participant 3	6.48	6.34	6.68	6.48
Participant 5	6.05	6.07	6.32	5.96
Participant 6	6.46	6.53	6.75	6.48
Participant 7	5.91	5.96	5.89	5.87
Participant 8	6.00	5.89	6.19	6.05
Participant 9	6.37	6.03	6.53	6.32
Participant 11	6.37	6.39	6.64	6.25
Participant 12	6.30	6.09	6.46	6.25
Participant 13	6.09	6.12	6.37	6.05
Participant 14	6.44	6.57	6.89	6.53
Participant 16	5.94	5.98	6.14	5.87
Participant 17	6.03	6.09	6.39	6.07
Participant 18	6.03	6.30	6.55	6.14
Participant 19	6.28	6.37	6.64	6.50
Participant 20	5.91	5.87	6.14	5.89
Participant 22	6.39	6.41	6.57	6.41
Participant 23	6.37	6.50	6.75	6.46

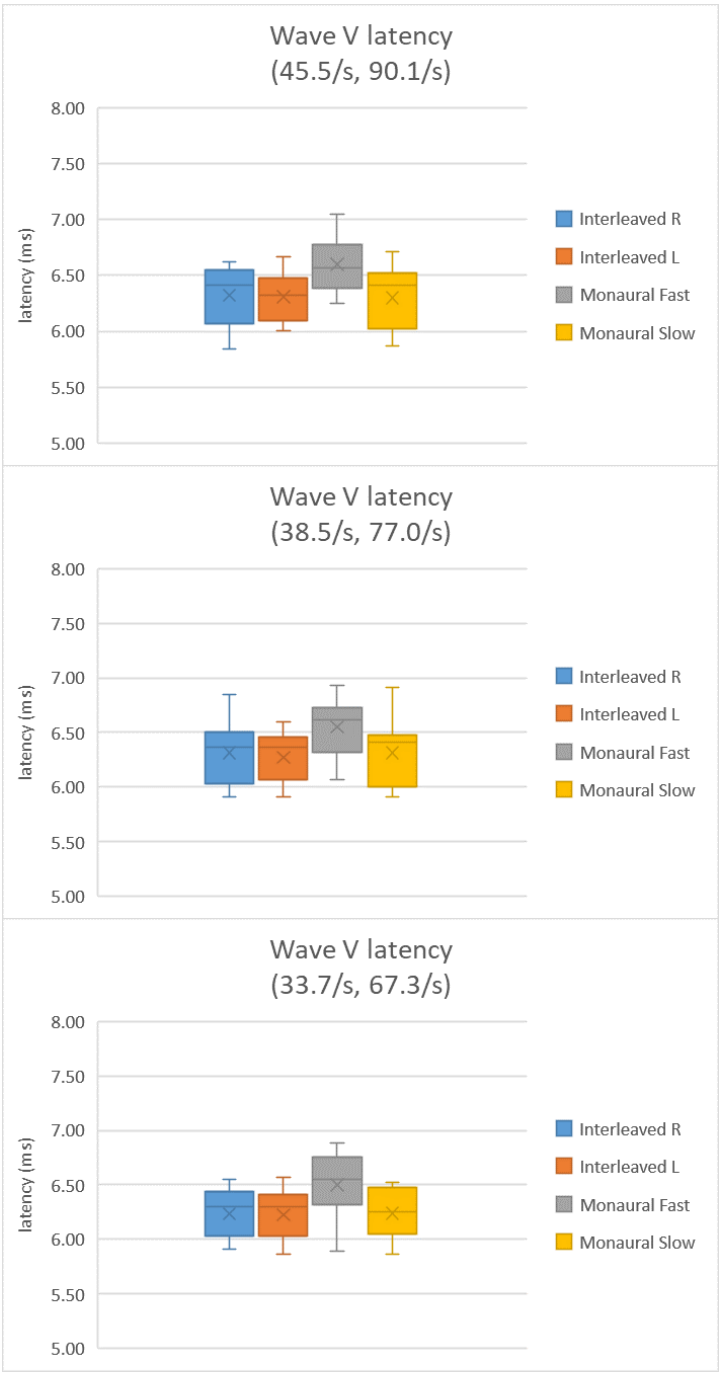

Figure 23. Box plots of wave V latency (ms) for the four recording conditions at three different rate combinations.

The box plots for each recording condition show the range between the minimum and maximum latencies with the shaded box area representing the interquartile range or middle

50% of each data set. The line inside each box marks the median value while the "X" marks the mean latency value. At each rate combination, the median and mean latency values for the Monaural fast recording condition are higher than the upper quartile value of the other recording conditions. As expected, the mean and median latency values of the left and right interleaved recording conditions are similar with the boxes mostly overlying each other. Furthermore, these two conditions appear to also be very similar to the Monaural Slow condition.

Table 6.

Wave V Latency Descriptive Statistics

	N	Minimum (ms)	Maximum (ms)	Mean (ms)	Std. Deviation	Skewness		Kurtosis	
						Statistic	Std. Error	Statistic	Std. Error
11/22 Right Interleaved	19	5.85	6.62	6.32	.25600	-.555	.524	-1.134	1.014
11/22 Left Interleaved	19	6.00	6.66	6.31	.22367	.101	.524	-1.384	1.014
11/22 Fast Monaural	19	6.25	7.05	6.60	.21976	.155	.524	-.758	1.014
11/22 Slow Monaural	19	5.87	6.71	6.30	.26463	-.194	.524	-1.458	1.014
13/26 Right Interleaved	19	5.91	6.84	6.31	.27447	.224	.524	-.959	1.014
13/26 Left Interleaved	19	5.91	6.59	6.27	.23049	-.223	.524	-1.403	1.014
13/26 Fast Monaural	19	6.07	6.93	6.55	.25149	-.280	.524	-1.050	1.014
13/26 Slow Monaural	19	5.91	6.91	6.31	.28017	.239	.524	-.622	1.014
15/30 Right Interleaved	19	5.91	6.55	6.24	.22566	-.194	.524	-1.620	1.014
15/30 Left Interleaved	19	5.87	6.57	6.23	.22687	-.093	.524	-1.426	1.014
15/30 Fast Monaural	19	5.89	6.89	6.50	.27513	-.582	.524	-.290	1.014
15/30 Slow Monaural	19	5.87	6.53	6.24	.24085	-.302	.524	-1.515	1.014

The minimum, maximum and mean (and standard deviation) wave V latency values are shown in Table 6. For each stimulus variable, all 19 participants with normal hearing were included. No significant skewness, kurtosis or significant outliers were present in any of the stimulus variable data sets. The assumptions of parametric testing were met.

3.1.1 Wave V latency 11/22 ms ISI. Mauchly's test of sphericity was not significant. $W(2) = .736$, $p = .074$. The assumption that the variances of the differences between all possible pairs of stimulus conditions are equal, has been met. Sphericity was assumed. An

analysis of variance (ANOVA) revealed that there was a significant main effect of stimulus. $F(2, 36) = 60.806$, $p < .001$, $\eta_p^2 = .772$. Posthoc testing showed that:

1. The wave V latency for the 11 ms ISI fast monaural testing condition was significantly longer than both the wave V latencies for the 11/22 ms ISI right interleaved and 22 ms ISI slow monaural testing conditions ($p < .001$). The null hypotheses were rejected.
2. The wave V latencies for the 11/22 ms ISI right interleaved and 22 ms ISI slow monaural testing conditions were not significantly different ($p = .326$). The null hypothesis was not rejected.

3.1.2 Wave V latency 13/26 ms ISI. Mauchly's test of sphericity was significant. $W(2) = .684$, $p = .039$. The assumption that the variances of the differences between all possible pairs of stimulus conditions are equal, has not been met. Sphericity was not assumed. Because Mauchly's test of sphericity was significant ($p < 0.05$) the assumption of sphericity is violated and the Greenhouse-Geisser correction was applied. An ANOVA revealed that there was a significant main effect of stimulus. $F(1.519, 27.347) = 42.975$, $p < .001$, $\eta_p^2 = .705$. Posthoc testing showed that:

1. The wave V latency for the 13 ms ISI fast monaural testing condition was significantly longer than both the wave V latencies for the 13/26 ms ISI right interleaved and 26 ms ISI slow monaural testing conditions ($p < .001$). The null hypotheses were rejected.
2. The wave V latencies for the 13/26 ms ISI right interleaved and 26 ms ISI slow monaural testing conditions were not significantly different ($p = .880$). The null hypothesis was not rejected.

3.1.3 Wave V latency 15/30 ms ISI. Mauchly's test of sphericity was not significant. $W(2) = .843$, $p = .233$. The assumption that the variances of the differences between all

possible pairs of stimulus conditions are equal, has been met. Sphericity was assumed. An ANOVA revealed that there was a significant main effect of stimulus. $F(2, 36) = 81.038$, $p < .001$, $\eta p2 = .818$. Posthoc testing showed that:

1. The wave V latency for the 15 ms ISI fast monaural testing condition was significantly longer than both the wave V latencies for the 15/30 ms ISI right interleaved and 30 ms slow monaural testing conditions ($p < .001$). The null hypotheses were rejected.
2. The wave V latencies for the 15/30 ms ISI right interleaved and 30 ms ISI slow monaural testing conditions were not significantly different ($p = .957$). The null hypothesis was not rejected.

The results above directly address the first three hypotheses in chapter 1.17. Firstly, the results confirm hypothesis 1, that the wave V latency measures for the Monaural Fast recording conditions are significantly different (longer) than the wave V latency measures for the Monaural Slow condition at all three rate combinations. Second, the wave V latency for the Monaural Fast condition are significantly different (longer) than the wave V latency for the Interleaved R at all three rate combinations. Lastly, there are no significant differences in wave V latency between the Interleaved R and Monaural Slow recording conditions.

3.2 Wave V Amplitude

The wave V amplitude values (μV pp) recorded and measured for each participant at all stimulus conditions are shown in Table 7, Table 8 and Table 9. These amplitude values are the amplitude of each of the two replicates of 3000 traces combined (i.e. 6000 traces).

Table 7.

Participant Wave V Amplitude (μV pp) Data for the 11/22 ms ISI Recordings

	11/22 ms ISI			
	Interleaved R	Interleaved L	Monaural Fast	Monaural Slow
Participant 1	0.54	0.42	0.52	0.48
Participant 2	0.50	0.54	0.40	0.56
Participant 3	0.34	0.49	0.37	0.42
Participant 5	0.54	0.59	0.42	0.78
Participant 6	0.65	0.47	0.67	0.56
Participant 7	0.51	0.40	0.50	0.60
Participant 8	0.40	0.47	0.49	0.30
Participant 9	0.51	0.36	0.58	0.50
Participant 11	0.24	0.27	0.17	0.26
Participant 12	0.40	0.50	0.46	0.44
Participant 13	0.32	0.48	0.47	0.38
Participant 14	0.26	0.16	0.09	0.39
Participant 16	0.77	0.78	0.61	0.84
Participant 17	0.42	0.55	0.37	0.43
Participant 18	0.23	0.26	0.36	0.36
Participant 19	0.19	0.22	0.15	0.25
Participant 20	0.53	0.41	0.66	0.65
Participant 22	0.40	0.46	0.51	0.43
Participant 23	0.44	0.32	0.39	0.46

Table 8.

Participant Wave V Amplitude (μV pp) Data for the 13/26 ms ISI Recordings

	13/26 ms ISI			
	Interleaved R	Interleaved L	Monaural Fast	Monaural Slow
Participant 1	0.40	0.37	0.48	0.43
Participant 2	0.46	0.45	0.43	0.51
Participant 3	0.35	0.40	0.44	0.43
Participant 5	0.56	0.53	0.61	0.47
Participant 6	0.54	0.54	0.41	0.50
Participant 7	0.48	0.49	0.57	0.47
Participant 8	0.40	0.37	0.31	0.30
Participant 9	0.60	0.50	0.51	0.52
Participant 11	0.22	0.30	0.10	0.22
Participant 12	0.37	0.39	0.47	0.49
Participant 13	0.42	0.49	0.45	0.48
Participant 14	0.29	0.46	0.39	0.29
Participant 16	0.73	0.79	0.63	0.77
Participant 17	0.44	0.53	0.34	0.40
Participant 18	0.34	0.38	0.38	0.33
Participant 19	0.19	0.26	0.23	0.17
Participant 20	0.71	0.66	0.61	0.67
Participant 22	0.39	0.44	0.52	0.30
Participant 23	0.44	0.42	0.29	0.29

Table 9.

Participant Wave V Amplitude (μV pp) Data for the 15/30 ms ISI Recordings

	15/30 ms ISI			
	Interleaved R	Interleaved L	Monaural Fast	Monaural Slow
Participant 1	0.39	0.37	0.50	0.52
Participant 2	0.44	0.38	0.46	0.43
Participant 3	0.26	0.41	0.31	0.34
Participant 5	0.52	0.27	0.36	0.59
Participant 6	0.43	0.48	0.58	0.50
Participant 7	0.33	0.37	0.32	0.50
Participant 8	0.34	0.41	0.37	0.33
Participant 9	0.51	0.47	0.59	0.48
Participant 11	0.27	0.20	0.16	0.26
Participant 12	0.44	0.43	0.45	0.43
Participant 13	0.54	0.62	0.43	0.46
Participant 14	0.37	0.34	0.43	0.23
Participant 16	0.77	0.76	0.65	0.73
Participant 17	0.41	0.49	0.37	0.42
Participant 18	0.37	0.47	0.37	0.41
Participant 19	0.16	0.15	0.15	0.17
Participant 20	0.77	0.54	0.70	0.57
Participant 22	0.47	0.43	0.53	0.28
Participant 23	0.45	0.37	0.39	0.36

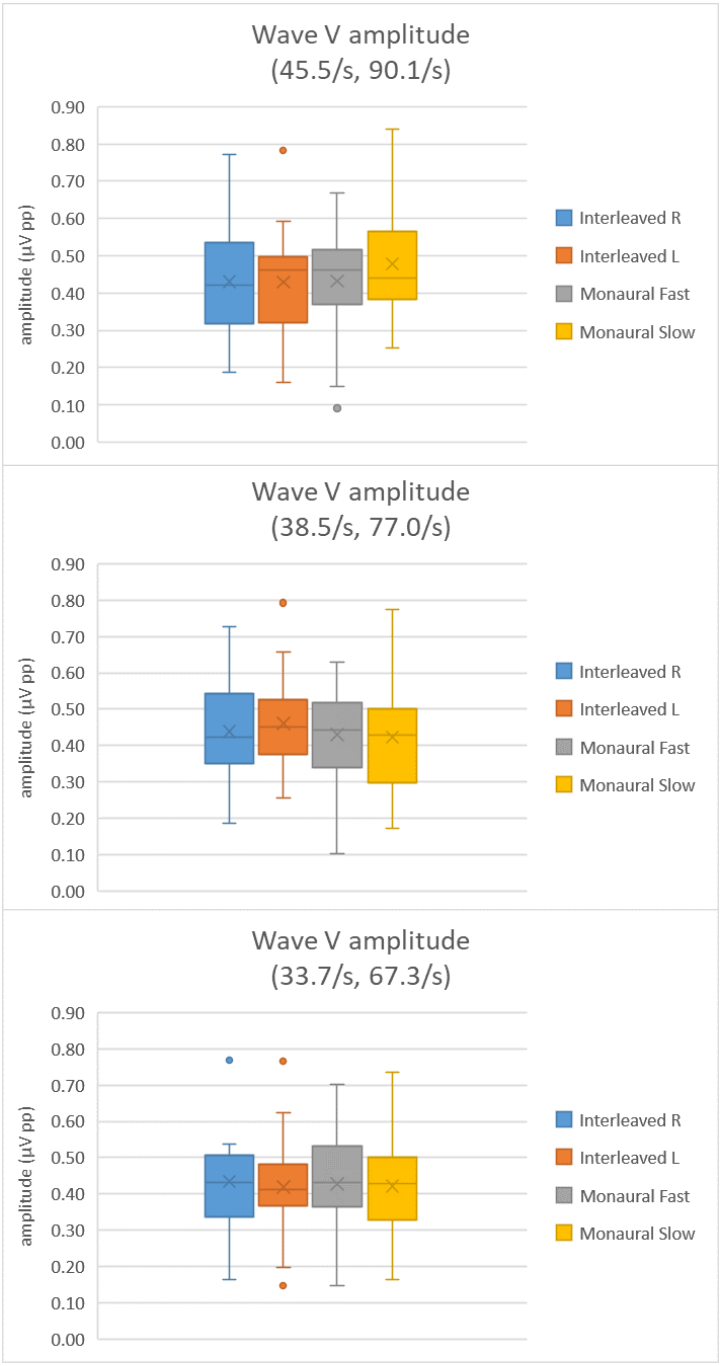

Figure 24. Box plots of wave V amplitude (μV pp) for the four recording conditions at three different rate combinations.

The box plots for each recording condition show the range between the minimum and maximum latencies with the shaded box area representing the interquartile range or middle 50% of each recording condition. The line inside each box marks the median value while the

"X" marks the mean latency value. Outliers are represented by dots outside the range of each box plot. However, none of these outliers are significant. For each rate combination, the box of all four recording conditions overlap and the mean and median amplitude values appear to be similar.

Table 10.

Wave V Amplitude Descriptive Statistics

	N	Minimum (μV pp)	Maximum (μV pp)	Mean (μV pp)	Std. Deviation	Skewness		Kurtosis	
						Statistic	Std. Error	Statistic	Std. Error
11/22 Right Interleaved	19	.19	.77	.43	.14937	.335	.524	.075	1.014
11/22 Left Interleaved	19	.16	.78	.43	.14522	.252	.524	.796	1.014
11/22 Fast Monaural	19	.09	.67	.43	.16110	-.655	.524	.129	1.014
11/22 Slow Monaural	19	.25	.84	.48	.15861	.791	.524	.464	1.014
13/26 Right Interleaved	19	.19	.73	.44	.14365	.435	.524	.151	1.014
13/26 Left Interleaved	19	.26	.79	.46	.12185	1.005	.524	2.000	1.014
13/26 Fast Monaural	19	.10	.63	.43	.13723	-.612	.524	.436	1.014
13/26 Slow Monaural	19	.17	.77	.42	.14844	.486	.524	.484	1.014
15/30 Right Interleaved	19	.16	.77	.43	.15174	.813	.524	1.298	1.014
15/30 Left Interleaved	19	.15	.76	.42	.13808	.384	.524	1.447	1.014
15/30 Fast Monaural	19	.15	.70	.43	.14541	-.072	.524	.033	1.014
15/30 Slow Monaural	19	.17	.73	.42	.13696	.193	.524	.195	1.014

The minimum, maximum and mean (and standard deviation) wave V amplitude values are shown in Table 10. For each stimulus variable, all 19 participants with normal hearing were included. No significant skewness or outliers were present in any of the stimulus variable data sets. There was significant kurtosis present for the 13/26 ms ISI left interleaved group. Because the main comparisons were between the right interleaved and the right fast monaural and right slow monaural groups, the kurtosis present in that group should not affect the statistical analyses.

3.2.1 Wave V amplitude 11/22 ms ISI. Mauchly's test of sphericity was significant. $W(2) = .543$, $p = .006$. The assumption that the variances of the differences between all

possible pairs of stimulus conditions are equal, has not been met. Sphericity was not assumed. Because Mauchly's test of sphericity was significant ($p < 0.05$) the assumption of sphericity is violated and the Greenhouse-Geisser correction was applied. An ANOVA revealed that there was no significant main effect of stimulus. $F(1.372, 24.700) = 2.320$, $p = .133$, $\eta_p^2 = .114$.

3.2.2 Wave V amplitude 13/26 ms ISI. Mauchly's test of sphericity was not significant. $W(2) = .863$, $p = .287$. The assumption that the variances of the differences between all possible pairs of stimulus conditions are equal, has been met. Sphericity was assumed. An ANOVA revealed that there was no significant main effect of stimulus. $F(2, 36) = .297$, $p = .745$, $\eta_p^2 = .016$.

3.2.3 Wave V amplitude 15/30 ms ISI. Mauchly's test of sphericity was not significant. $W(2) = .846$, $p = .241$. The assumption that the variances of the differences between all possible pairs of stimulus conditions are equal, has been met. Sphericity was assumed. An ANOVA revealed that there is no significant main effect of stimulus. $F(2, 36) = .137$, p .873, $\eta_p^2 = .008$.

The results above address hypothesis 4-6 in chapter 1.17. Hypotheses 4 and 5 are disproven as there are no significant differences in wave V amplitude between all recording conditions at all three rate combinations. Hypothesis 6 stands as there is no significant difference in amplitude between Interleaved R and Monaural Slow recordings at all three rate combinations.

3.3 Number of Sweeps Required to Reach Fsp of 3.1

The average number of sweeps (stimuli presented) were recorded and measured for each participant at all stimulus conditions are shown in Table 11, Table 12 and Table 13.

Table 11.

Average Number of Recordings Required to Reach an Fsp of 3.1 for the 11/22 ms ISI Recordings for Each Participant

	11/22 ms ISI			
	Interleaved R	Interleaved L	Monaural Fast	Monaural Slow
Participant 1	805.00	2528.50	677.50	525.50
Participant 2	2315.50	1279.00	823.50	421.00
Participant 3	2037.00	1053.50	1874.00	1731.00
Participant 5	1483.00	1821.00	1565.00	1286.50
Participant 6	729.00	1150.50	257.00	152.50
Participant 7	294.50	795.50	1106.00	1292.50
Participant 8	1457.00	959.50	1306.00	2349.00
Participant 9	2638.00	*4000.00*	1784.50	2241.00
Participant 11	808.50	1012.00	961.00	2260.50
Participant 12	1710.50	1439.50	*4000.00*	2006.00
Participant 13	2693.00	2467.00	1491.50	2513.00
Participant 14	1559.00	*4000.00*	1918.00	759.50
Participant 16	263.00	474.00	337.50	316.50
Participant 17	1434.50	987.50	1797.00	1196.50
Participant 18	519.00	565.00	2460.00	1610.50
Participant 19	*4000.00*	*4000.00*	*4000.00*	1486.50
Participant 20	*4000.00*	383.00	2011.00	600.00
Participant 22	2382.00	*4000.00*	*4000.00*	*4000.00*
Participant 23	1978.00	2534.00	2017.50	1313.00

Table 12.

Average Number of Recordings Required to Reach an Fsp of 3.1 for the 13/26 ms ISI Recordings for Each Participant

	13/26 ms ISI			
	Interleaved R	Interleaved L	Monaural Fast	Monaural Slow
Participant 1	1886.00	1483.50	2168.50	1358.00
Participant 2	1042.00	835.50	634.50	606.50
Participant 3	1610.50	2210.50	2207.50	1267.00
Participant 5	*4000.00*	*4000.00*	2079.00	2483.00
Participant 6	190.00	148.50	337.00	285.00
Participant 7	1846.50	1242.50	1196.50	1429.50
Participant 8	*4000.00*	1926.50	2576.00	2404.00
Participant 9	1200.50	1268.50	582.50	573.00
Participant 11	2084.00	1840.00	*4000.00*	1793.00
Participant 12	2124.00	1723.00	1251.00	851.50
Participant 13	1569.50	1402.50	1732.00	1744.00
Participant 14	635.50	1166.00	1261.50	526.00
Participant 16	414.50	378.00	1019.50	442.50
Participant 17	1230.50	1036.00	2068.00	747.50
Participant 18	*4000.00*	*4000.00*	2501.00	1679.50
Participant 19	1990.00	*4000.00*	2140.00	2388.00
Participant 20	1619.50	1601.00	2446.00	2260.00
Participant 22	1755.00	1274.00	2349.00	2678.00
Participant 23	2169.00	808.00	*4000.00*	*4000.00*

Table 13.

Average Number of Recordings Required to Reach an Fsp of 3.1 for the 15/30 ms ISI Recordings for Each Participant

	15/30 ms ISI			
	Interleaved R	Interleaved L	Monaural Fast	Monaural Slow
Participant 1	1111.50	1466.50	1920.50	1276.00
Participant 2	1426.00	990.00	2610.00	1131.00
Participant 3	2382.00	2373.00	*4000.00*	*4000.00*
Participant 5	*4000.00*	*4000.00*	*4000.00*	*4000.00*
Participant 6	477.50	515.00	448.00	281.50
Participant 7	1212.00	2102.50	1381.00	566.50
Participant 8	*4000.00*	2068.50	2215.00	1959.50
Participant 9	1767.00	2278.50	1524.00	2315.00
Participant 11	1337.00	1495.50	*4000.00*	2721.00
Participant 12	835.00	1669.00	*4000.00*	1225.50
Participant 13	361.00	1925.00	2323.50	1521.00
Participant 14	689.00	583.00	980.50	761.00
Participant 16	902.50	428.50	823.50	204.50
Participant 17	743.00	808.00	653.00	522.00
Participant 18	*4000.00*	*4000.00*	1061.00	264.50
Participant 19	*4000.00*	*4000.00*	1535.00	1456.00
Participant 20	2257.00	2968.00	1474.00	2174.00
Participant 22	2122.00	1864.00	*4000.00*	1722.00
Participant 23	1640.00	*4000.00*	*4000.00*	*4000.00*

In Table 11, Table 12 and Table 13, all values which are exactly 4000.00 are recordings which failed to reach an Fsp of 3.1. A value of 4000.00 is used to represent these missing data points.

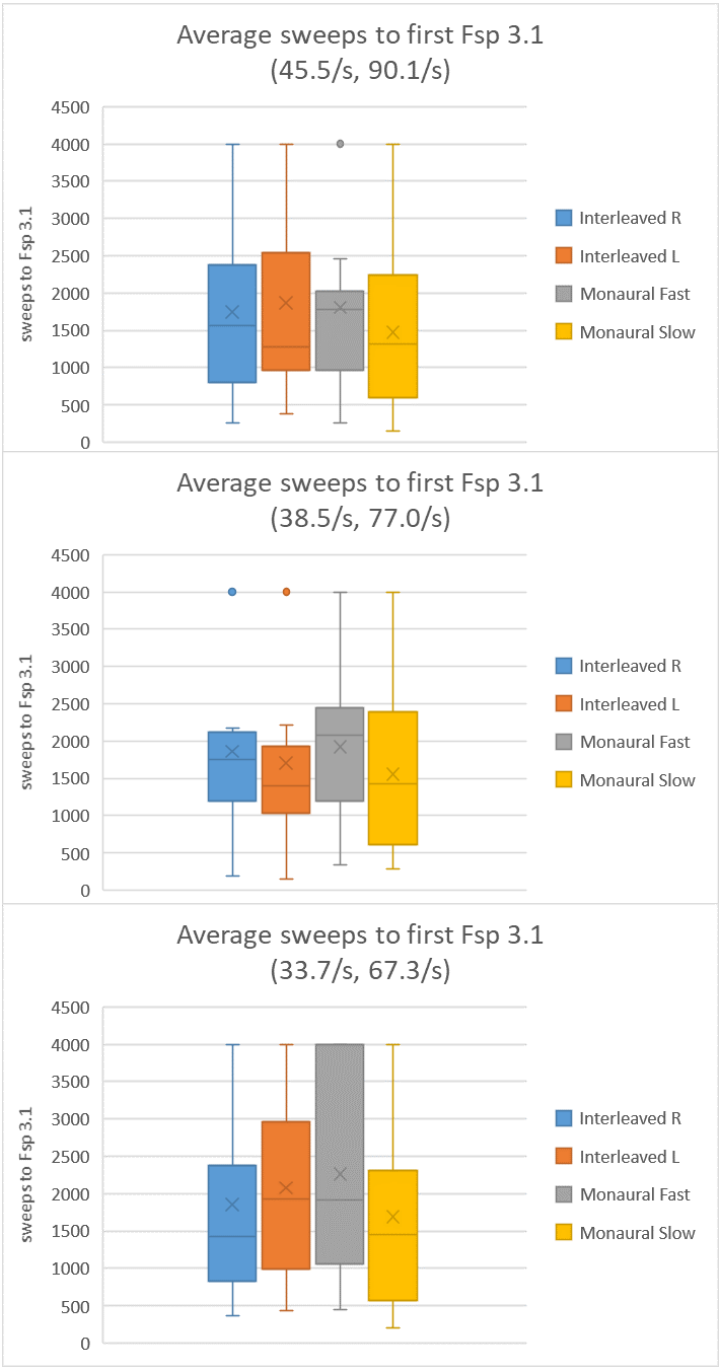

Figure 25. Box plots of average number of recordings required to reach an Fsp of 3.1 for the four recording conditions at three different rate combinations.

The box plots for each recording condition show the range between the minimum and maximum latencies with the shaded box area representing the interquartile range or middle 50% of each data set. The line inside each box marks the median value while the "X" marks

the mean latency value. Outliers are represented by dots outside the range of each box plot. However, none of these outliers are significant. The maximum value or maximum outliers for all of the recording conditions are at a value of 4000 sweeps as these represent data which never reached and Fsp of 3.1 as discussed above. For each rate combination, the box of all four recordings settings overlap but the mean and median value for the monaural fast condition appear to be higher compared to the other 3 recording conditions.

Table 14.

Descriptive Statistics for the Average Number of Recordings Required to Reach an Fsp of 3.1

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
						Statistic	Std. Error	Statistic	Std. Error
11/22 Right Interleaved	19	263.00	4000	1742.45	1095.87	.671	.524	.071	1.014
11/22 Left Interleaved	19	383.00	4000	1865.76	1300.88	.764	.524	-.893	1.014
11/22 Fast Monaural	19	257.00	4000	1809.84	1139.65	.895	.524	.253	1.014
11/22 Slow Monaural	19	152.50	4000	1476.89	952.79	.856	.524	1.218	1.014
13/26 Right Interleaved	19	190.00	4000	1861.42	1107.19	.856	.524	.457	1.014
13/26 Left Interleaved	19	148.50	4000	1702.32	1138.91	1.192	.524	.772	1.014
13/26 Fast Monaural	19	337.00	4000	1923.66	1008.83	.506	.524	.268	1.014
13/26 Slow Monaural	19	285.00	4000	1553.47	976.89	.749	.524	.437	1.014
15/30 Right Interleaved	19	361.00	4000	1855.92	1270.62	.844	.524	-.622	1.014
15/30 Left Interleaved	19	428.50	4000	2080.79	1221.14	.458	.524	-.861	1.014
15/30 Fast Monaural	19	448.00	4000	2260.47	1330.65	.336	.524	-1.519	1.014
15/30 Slow Monaural	19	204.50	4000	1689.53	1253.10	.800	.524	-.296	1.014

The minimum, maximum and mean (and standard deviation) average number of recordings to reach an Fsp of 3.1 are shown in Table 14. For each stimulus variable, all 19 participants with normal hearing were included. No significant kurtosis or outliers were present in any of the stimulus variable data sets. There was significant skewness present for the 13/26 ms ISI left interleaved group. Because the main comparisons made were between the right interleaved and the right fast monaural and right slow monaural groups, the skewness present in that group should not affect the statistical analyses.

3.3.1 Sweeps 11/22 ms ISI. Mauchly's test of sphericity was not significant. $W(2) = .897$, $p = .397$. The assumption that the variances of the differences between all possible pairs of stimulus conditions are equal, has been met. Sphericity was assumed. An ANOVA revealed that there was no significant main effect of stimulus. $F(2, 36) = .930$, $p = .404$, $\eta_p^2 = .049$.

3.3.2 Sweeps 13/26 ms ISI. Mauchly's test of sphericity was not significant. $W(2) = .762$, $p = .099$. The assumption that the variances of the differences between all possible pairs of stimulus conditions are equal, has been met. Sphericity was assumed. An ANOVA revealed that there was no significant main effect of stimulus. $F(2, 36) = 1.844$, $p = .173$, $\eta_p^2 = .093$.

3.3.3 Sweeps 15/30 ms ISI. Mauchly's test of sphericity was significant. $W(2) = .623$, $p = .018$. The assumption that the variances of the differences between all possible pairs of stimulus conditions are equal, has not been met. Sphericity was not assumed. Because Mauchly's test of sphericity was significant ($p < 0.05$) the assumption of sphericity is violated and the Greenhouse-Geisser correction was applied. An ANOVA revealed that there was no significant main effect of stimulus. $F(1.452, 26.136) = 1.733$, $p = .201$, $\eta_p^2 = .088$.

Similar to wave V amplitude, the results show that there are no significant differences in the number of sweeps required between all four recording conditions at all three rate combinations. This disproves hypotheses 7 and 8 and confirms hypothesis 9.

Chapter 4: Discussion

The aim of the current study was to determine if using interleaved stimuli presented to the left and right ears would not only allow the recording of ABR data at twice the rate of recording monaurally from the left and right ears sequentially but also if it would yield waveforms that were of equal quality as monaurally presented stimuli. It was hypothesized that the wave V latency, wave V amplitude, and sweeps required to reach an Fsp of 3.1 measured for the interleaved stimuli would not be significantly different to the Monaural Slow condition but would be significantly different to the Monaural Fast condition.

4.1 Experiment Setup and Design

The study had been intended to be carried out with chirp stimuli, but technical issues led to the use of click stimuli for all the ABR recordings. Additionally, because click stimuli are one of the more commonly used stimuli in clinical practice, it would make findings from this study more clinically applicable. Furthermore, click stimuli yields generally clearer waveforms compared to frequency specific tone-bursts as it activates a larger part of the auditory system.

The stimuli for all recording conditions were presented at 70 dB nHL, this level was appropriate because it was a level sufficiently loud to produce clear results but was not too loud to cause discomfort for each participant to listen to almost continuously throughout each testing. Each participant was instructed that they can choose to stop the testing anytime and to inform the investigator if they felt discomfort at any time during the experiment.

The stimulus rates were chosen such that they ranged from those that are commonly used clinically, to those that result in compromised waveform morphology and prolonged wave V latencies. In particular, the 45.5/s and 90.1/s combination were chosen in the hope of maximising this difference in waveform quality.

The electrode montage chosen was the vertical montage instead of the more conventional ipsilateral electrode montage. This decision was made because not only does the vertical montage produce larger wave V amplitudes compared to the ipsilateral montage (Dzulkarnain, Wilson, Bradley & Petoe, 2008; Sininger, 1992) but it also made data wrangling and the organization of the results easier, and required fewer electrodes, saving resources and preparation time before testing.

Wave III peaks and latencies were also identified during the data analysis and potentially could have been included in the data analysis to complement the wave V data analysis. The identification of the wave III peaks was often difficult as these peaks were not as clearly present as the wave V peaks. Consequently, this led to the primary investigator having to approximate where they expected the wave III to be. This made the latency and amplitude data obtained for wave III less reliable and it was decided to exclude these data from data analysis and rely primarily on the more reliable and consistent wave V data. The vertical electrode montage chosen for this study may have contributed to these results as employing this electrode montage leads to higher larger wave V peaks but smaller wave I and III compared to the ipsilateral montage (Dzulkarnain, Hadi & Zakaria 2013; Dzulkarnain et al., 2017).

4.2 Discussion of Results

The latency, amplitude and other measures evaluated in the Results section are obtained by adding together the two replicate traces of approximately 3000 traces each then using the values from the resulting 6000 average trace. ANOVA testing was conducted for each measure to determine if there was a significant effect of the testing condition in general while post-hoc testing was completed comparing the Interleaved R, Monaural Fast and Monaural Slow conditions. Only the Interleaved R condition was compared to the Monaural conditions as the Monaural testing conditions were completed on the right ear. It was

appropriate to compare ABR results conducted on the same ear as thresholds may vary between the two ears for any participant.

4.2.1 Wave V latency. Figure 23 shows a series of box plots comparing the mean latency of the four recording conditions at all three rate combinations. As hypothesized, the three sets of ANOVA results show that the Monaural Fast condition produces significantly longer wave V latencies compared to the Monaural Slow and Interleaved R conditions. There are no significant latency differences between the Interleaved R and Monaural Slow conditions. The hypotheses were produced under the overlying assumption that the adaptation of the ascending auditory pathway is confined peripherally and that the interleaved stimuli activate separate sets of neurons. This assumption is supported by literature (Don, Allen & Starr, 1977; Eggermont & Odenthal, 1974). The latency results support these hypotheses. Firstly, it was expected that the Wave V latencies are significantly longer for the Monaural Fast compared to the Monaural Slow conditions at all rate combinations. With all other recording parameters kept constant, monaural stimuli presented at a higher rate (90.9/s, 76.9/s and 66.7/s) produced shorter wave V peak latencies compared to stimuli presented at a slower rate (45.5/s, 38.5/s and 33.3/s respectively). This result is consistent with the rate induced adaptation effect well-established in literature (Don, Allen & Starr, 1977; Fowler & Noffsinger, 1983; Gerling & Finitzo-Hieber, 1983; Hyde, Stephens & Thornton, 1976; Jewett & Williston, 197; Pratt & Sohmer, 1976; Weber & Fujikawa, 1977). The Interleaved R condition produced significantly shorter latencies than the wave V while not being significantly different to the latencies of the Monaural Slow condition. These results are consistent with the assumptions and hypotheses of this study. It suggests that ABR waveforms do not demonstrate fatigue or exhibit adaptation effects (in terms of wave V latency) when interleaved stimuli are presented at an overall rate that would cause fatigue when presented monaurally. Similarly, interleaved stimuli produce similar wave V latencies

for a stimulated ear as it would if the contralateral ear was not also being stimulated. That is, the latency is dependent on the peripheral rate. If the adaptation effects were to stem centrally, then each individual stimulus being presented regardless of which ear, would compound on the effects of the previous stimulus and lead a higher level of adaptation or fatigue compared to if the fatigue effects were peripheral. In this central adaptation scenario, it would be expected for the Interleaved R condition to exhibit similar adaptation effects as the Monaural Fast condition and therefore show similar latencies. The Interleaved R condition should also show significantly longer wave V latencies compared to the Monaural Slow condition. Because the results are the opposite to the expected results for the central adaptation scenario, the observed results support the assumption that adaptation occurs peripherally.

4.2.2 Wave V amplitude. The results of the ANOVA for the wave V amplitudes show that there are no significant differences in wave V amplitude between all stimulus conditions at all three rate combinations. It was expected for the Monaural Fast conditions to show reduced wave amplitude in comparison to the Slow monaural condition due to adaptation, similar to the results observed with latency above. To see no significant differences in amplitude between these two groups is an unexpected result. Perhaps this result may occur if higher rate combinations were tested. A key finding however, was that the amplitude for the Interleaved R group was not significantly different (i.e. higher) than the amplitude of the Monaural Slow group at all rate combinations. This result combined with the results from the latency measures again suggest that there is no increased effect of adaptation when using interleaved stimuli compared to if these stimuli were presented at the same rate individually to one ear at a time.

4.2.3 Number of sweeps required to reach Fsp of 3.1. During each ABR recording, the Fsp was recorded to indicate the SNR and thus as an objective measure of the quality of

each waveform. The final Fsp value reached after approximately 3000 presentations (at each stimulus condition, the recording stops after approximately 3000 sweeps) were measured as well as the number of sweeps that is required to reach an Fsp of 3.1. Fewer sweeps required to reach an Fsp of 3.1 indicate that it is a cleaner or better-quality waveform compared to a recording that takes many sweeps before it reaches 3.1 Fsp. Careful considerations were made to ensure that the Fsp reached were as high as possible. For some recordings, Fsp of 3.1 were not reached before the recording stopped after 3000 sweeps, characteristic to a poorer waveform. This shows up as an absent value in the recorded data as there as there is no point at which the Fsp reaches 3.1. It is acknowledged that any method to remedy this absence of data will result in some bias in the statistical analyses. It was decided that these absent Fsp values would be replaced with an arbitrarily high number (4000) to represent these waveforms. This value of 4000 to represent the absent data was chosen as it indicates a relatively "poor" waveform that would have taken 4000 sweeps before reaching an Fsp of 3.1. Some of these absent data may have potentially reached an Fsp of 3.1 just after stopping at 3000 sweeps (e.g. around 3100 sweeps) while others may have taken a very high number of sweeps before ever reaching an Fsp of 3.1 (e.g. 5000 sweeps, or perhaps never). Choosing a standard number to represent these absent data may be less biased than choosing a different number for each recording based on the projection of each live Fsp recording. Alternatively, each absent data point may have been removed instead. However, not only does this still bias the data, but it will also lose statistical power as there were a few missing data points. In Table 11, Table 12 and Table 13 and for the rest of the analysis, the missing data points have been replaced by a value of "4000.00".

There were no significant differences in the number of sweeps required to reach Fsp of 3.1 between all stimulus conditions at all three rate combinations. It was hypothesized that there would be a significant difference in the number of sweeps between the Monaural Fast

and Monaural Slow conditions (more sweeps required for the Monaural Fast condition) however, the results did not show this. The Interleaved R and Monaural Slow values were not significantly different at all rate combinations again suggesting that there is no increased effect of adaptation and fatigue when using interleaved stimuli compared to if these stimuli were presented individually at the same rate to one ear at a time. This result is not unexpected in light of the amplitude data- Fsp is a measure of SNR, and the wave V amplitude (a main determinant of the "signal" component of the SNR). Therefore, if the noise did not change between recording conditions, we would not expect the SNR or Fsp to show differences.

4.4 Study Limitations

One limitation of this study is the relatively small sample size of 19 participants which was largely due to time limitations. Strong conclusions based on the findings from this data may be limited when generalizing to the wider population. Furthermore, this testing was performed in a controlled environment with low electrical and acoustic noise present, this would be different in a clinical setting such as in a hospital so testing in those environments may differ.

During testing, many variables were difficult to control. While random, transient background noise was minimized during testing and effects were reduced by signal averaging, longer periods of increased background noise was difficult to account for (e.g. planes flying nearby, people talking in a nearby room). The 70 dB nHL level was chosen because it was loud enough to produce a desirable SNR yet comfortable enough for participants to tolerate the whole duration of testing. Muscle tension and overall relaxation state of the participant heavily affected the Fsp measurement. Moreover, the participants appeared to be progressively more relaxed as the testing proceeded. Initially they would produce lower Fsp as the test commenced then would slowly relax or even fall asleep midway or near the end of testing. Because all participants were tested in the order of 11/22,

13/26 then 15/30 ms ISI rate combinations, it could potentially mean that the 13/26 ms ISI and 15/30 ms ISI rate combinations produced higher Fsp values compared to the earlier 11/22 ms ISI rate combination because the participants were more relaxed. Additionally, within each rate combination, the Interleaved condition was tested first followed by the Monaural Fast and finally Monaural Slow conditions. Especially for the 11/22 ms ISI rate combination, the Fsp of the interleaved condition may have been worse compared to the other conditions because it was the first parameter tested and the participant may have still been in the process of fully relaxing. However, the assumption that the participants were progressively relaxed as the testing occurred may not apply to all the participants, their muscle tension and relaxation may have fluctuated several times during the duration of the testing. Potentially, some participants may have even been progressively tense as the test went on as they became more anxious. This problem could have been accounted for by randomly choosing the order of the rate combination and test condition that was presented to each participant. However, this would have required for a different pre-programmed sequence of test setting to be made prior to testing each participant. Consideration should be given to including a randomising feature to the sequencer module.

The analysis of the waveform results was completed by the primary investigator. Though care was taken to closely examine and mark the boundaries of each wave V and each analysis was double checked by the primary supervisor, this non-blinded visual inspection of the results could have potentially been a source of bias. An alternative method could be to have an independent examiner to mark the peak boundaries.

The wave V peaks of the waveforms were easily identifiable, but it was at times difficult to determine the end boundary of the wave V as they merged with the succeeding wave VI, forming a wave V-VI complex. This pattern was observed mostly for the Monaural Fast recording condition potentially because greater effects of adaptation in this recording

condition caused the waveform shape to deteriorate and lead to these two peaks merging. This dilemma has been acknowledged in literature and one way that this has been addressed was to measure the amplitude of wave V from its peak to the trough of the following wave VI (Gu, Herrmann, Levine & Melcher, 2012). It was not appropriate to apply this method for the waveforms with merged wave V and wave VI as it would bias the data and make these few waveforms have larger amplitudes compared to waveforms without these merged complexes. Furthermore, because most of the waveforms had distinct wave V borders, it was decided that it was unnecessary to apply this measurement method to all waveforms as this would skew the wave V amplitudes to be generally larger. The approach used in this study for these few waveforms was to estimate where the boundary of the wave V as if the wave V/VI complex was not present and mark the boundaries accordingly. Bias was minimized by estimating and marking the boundaries as consistently as possible and setting the boundaries using the other waveforms obtained by that participant as a guide. The assumption of sphericity was not met for some ANOVA test groups. For these groups, the Greenhouse-Geisser correction was applied.

4.4 Future Research and Applications

It is necessary for future research to be directed towards further investigating the effects of interleaved click stimuli but with a larger sample size to increase the power of the study and enable stronger generalizations regarding the wider population. Additionally, variations from this study that could provide more information regarding interleaved stimuli include testing at a different intensity level or at different rate combinations. Once, multiple studies on normal hearing participants have produced consistent results, testing on other groups such as children, or adults with conductive or sensorineural hearing loss may be of interest. Understanding the neurophysiologic development in neonatal ABR responses and how the newborn auditory system responds to high stimulus rates is important as findings

from this research may be useful and applicable in the paediatric population. Especially when considering neonatal hearing screening programmes and the use of ABR in paediatric populations, the time-saving and convenience advantages that the proposed presentation paradigm may provide could have huge implications for the refinement of protocols currently in place. For any ABR technique to be used in paediatric subjects, it should first be safely trialled in normal hearing adults before being further investigated in paediatric subjects. The results from this paper, based on normal hearing adults may become the foundation for future research on impaired hearing and/or paediatric subjects.

The multi-channel ABR recording software developed for this study is not only capable of interleaving two click stimuli between the ears but also multiple different stimuli at varied, rates, intensity and stimulus types such as tone-bursts. A future study may be interested in employing a similar research design as this paper but investigate the characteristic effects of interleaving frequency specific stimuli such as tone-bursts or chirps instead. This will require careful planning and rational reasoning for choosing the frequency and intensity order of the stimuli presented to ensure adaptation effects are minimized. A research group may even focus on quantifying and evaluating in detail the practical time-saving advantages of using interleaved stimuli both in a laboratory and clinical setting. There is a vast avenue of potential research designs that could stem from the use of the ABR software used in this study to study interleaved stimuli.

Since the interleaved presentation method proposed in this paper was designed to obtain ABR waveforms evoked by multiple intensities near-simultaneously, it can provide advantages in constructing latency-intensity functions. By simultaneously presenting interleaved stimuli at different intensity levels, it is possible to construct a latency-intensity function from one set of recordings recorded under identical conditions, minimising the effects of external factors (e.g. muscle tension) that may affect the ABR being recorded at

different or over prolonged time periods. Such an approach has been taken in this laboratory previously when recording vestibular evoked myogenic potential (VEMP) waveforms (Johnson et al., 2016).

4.5 Conclusions

The interleaving of stimuli to record the ABR allows for the reduction of overall test time compared to the conventional sequential method of recording. This is potentially valuable in a clinical setting by enabling clinicians to complete a more thorough diagnostic testing within their allocated time. This advantage is only beneficial if interleaved stimuli still yields quality waveforms, comparable to their monaural counterparts. Results from this study revealed that ABR waveforms obtained using two interleaved click stimuli at 70 dB nHL have wave V amplitudes, wave V latencies and Fsp measures that are comparable to ABR waveforms obtained using monoaural stimuli at 70dB nHL, at an equivalent rate to each interleaved stimulus. This was the case at three different sets of rates. Furthermore, results showed that this interleaved stimulus produce significantly shorter latencies than monaural stimuli presented at the combined rate of the interleaved stimuli. These results are consistent with the underlying assumption that the adaptation mechanisms of these interleaved stimuli occur peripherally and therefore do no not fatigue the same neurons centrally in the same manner as simply increasing the rate of a monaural stimulus.

Chapter 5: References

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Chapter 6: Appendices

Appendix A: Ethics Approval

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

HUMAN ETHICS COMMITTEE

Secretary, Rebecca Robinson
Telephone: +64 03 369 4588, Extn 94588
Email: human-ethics@canterbury.ac.nz

Ref: HEC 2019/17/LR

30 May 2019

Steven Landel Pastrana Bencito
Communication Disorders
UNIVERSITY OF CANTERBURY

Dear Steven

Thank you for submitting your low risk application to the Human Ethics Committee for the research proposal titled "Interleaved Recording of the Auditory Brainstem Response".

I am pleased to advise that this application has been reviewed and approved.

Please note that this approval is subject to the incorporation of the amendments you have provided in your emails of 21st and 24th May 2019.

With best wishes for your project.

Yours sincerely

R. Robinson

pp.

Professor Adrian Sawyer
Deputy Chair, Human Ethics Committee

Appendix B: Information Sheet

Department of Communication Disorders
Telephone: +64 33694313

Email: stevenlandel.bencito@pg.canterbury.ac.nz

17 May 2019
HEC Ref: 2019/17/LR

Interleaved Recording of the Auditory Brainstem Response

Information Sheet for Persons Participating in Research Studies

My name is Steven Bencito, I am a 2nd year Master of Audiology student conducting research on the auditory brainstem response. This study is being performed with the goal of determining if using an interleaved method of presenting stimuli in auditory brainstem response recordings will offer practical advantages in terms of response quality, test time, and diagnostic accuracy.

You have been approached to take part in this study because you are over 18 years old, have normal hearing and is able to have the auditory brainstem response recorded.

If you choose to take part in this study, your involvement in this project will be getting asked for a history of your ear health and hearing and have your ears examined. You will then have a hearing test. In the event of an unexpected diagnosis of a hearing loss, a full audiological assessment will be offered at the University of Canterbury Speech and Hearing Clinic free of charge. If you choose to follow up with your GP, this will, however, be at your own expense.

Following the hearing test, the rest of the study will take place. You will sit in a comfortable position and relax. While you are sitting comfortably, we will measure tiny electrical signals from your scalp that are produced by the brain in response to sound (the "auditory brain-stem response"). Using a tissue and some cleaning alcohol, we will first lightly exfoliate the skin where the adhesive sensors will be placed to make sure they can pick up the tiny signals. Sounds will be played through earphones placed on both ears while we record the signals. After testing, the sensors will be carefully removed, and the session will be finished.

In the performance of the tasks and application of the procedures there are risks of emotional distress which is no greater than the risk any adult would normally experience when consulting for hearing services. The procedures in this study are the same procedures a client would normally encounter in a hearing evaluation. When cleaning and preparing electrode sites the participants' skin is lightly exfoliated, which can occasionally cause these areas to be reddened. Alcohol hand cleaner will also be used, which can sometimes cause skin irritation, but if this occurs, a soothing cream will be provided.

Participation is voluntary and you have the right to withdraw at any stage without penalty. You

may ask for your raw data to be returned to you or destroyed at any point. If you withdraw, I will remove information relating to you. However, once analysis of raw data starts on 1 August 2019, it will become increasingly difficult to remove the influence of your data on the results.

The results of the project may be published, but you may be assured of the complete confidentiality of data gathered in this investigation: your identity will not be made public without your prior consent. To ensure anonymity and confidentiality, participants will be assigned an ID number. Data (hearing history sheet, hearing test results, speech perception test results) will contain only the participant ID. The data will be stored securely and may only be accessed by the primary researcher and thesis supervisors. Identifying information (consent forms, release of information forms, and requests for study results) will be stored securely and separately from the data. The stored data will be destroyed after 5 years. A thesis is a public document and will be available through the UC Library.

Please indicate to the researcher on the consent form if you would like to receive a copy of the summary of results of the project.

The project is being carried out as a requirement for a Master of Audiology thesis by Steven Bencito under the supervision of Greg o'Beirne, who can be contacted at gregory.obeirne@canterbury.ac.nz. He will be pleased to discuss any concerns you may have about participation in the project.

This project has been reviewed and approved by the University of Canterbury Human Ethics Committee, and participants should address any complaints to The Chair, Human Ethics Committee, University of Canterbury, Private Bag 4800, Christchurch (human-ethics@canterbury.ac.nz).

If you agree to participate in the study, you are asked to complete the consent form and return to Steven Bencito, contacted through email at (stevenlandel.bencito@pg.canterbury.ac.nz)

Appendix C: Consent Form

Department of Communication Disorders
Telephone: +64 2102692145
Email:
stevenlandel.bencito@pg.canterbury
.ac.nz

Interleaved recording of the Auditory Brainstem Response

Consent Form for Persons Participating in Research Studies

- ☐ I have been given a full explanation of this project and have had the opportunity to ask questions.
- ☐ I understand what is required of me if I agree to take part in the research.
- ☐ I understand that participation is voluntary and I may withdraw at any time without penalty. Withdrawal of participation will also include the withdrawal of any information I have provided should this remain practically achievable.
- ☐ I understand that any information or opinions I provide will be kept confidential to the researcher and their primary supervisor and that any published or reported results will not identify the participants. I understand that a thesis is a public document and will be available through the UC Library.
- ☐ I understand that all data collected for the study will be kept in locked and secure facilities and/or in password protected electronic form and will be destroyed after five years.
- ☐ I understand the risks associated with taking part and how they will be managed.
- ☐ I understand that I can contact the researcher Steven Bencito (stevenlandel.bencito@pg.canterbury.ac.nz) or supervisor Greg O'Beirne (gregory.obeirne@canterbury.ac.nz) for further information. If I have any complaints, I can contact the Chair of the University of Canterbury Human Ethics Committee, Private Bag 4800, Christchurch (human-ethics@canterbury.ac.nz)
- ☐ I would like a summary of the results of the project.
- ☐ By signing below, I agree to participate in this research project.

Name:_____ Signed:_____ Date:_____

Email address *(for report of findings, if applicable)*:

_____

Please return form to Steven Bencito (stevenlandel.bencito@pg.canterbury.ac.nz)

Appendix D: Recruitment Advertisement

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

Email Invitation

Hi everyone,

VOLUNTEERS NEEDED!

We are developing a new way to monitor hearing. This test will use two earphones on either ear. We will play sounds through these two speakers and measure tiny electrical signals from your skin. We will measure responses from two different types of signals: one ear at a time and both ears at the same time.

If you are:

- are 18 years of age or older
- have normal hearing

Then we would like to hear from you!

This study will take place at the University of Canterbury Speech and Hearing Clinic throughout 2019.

You would be needed for one 2 hour session, during this time you will:

- receive a free hearing test
- help to develop a new hearing monitoring technique
- receive a $20 fuel voucher as a token of our appreciation.

For more information, or to be involved in this project, please contact **Steven Bencito** at **stevenlandel.bencito@pg.canterbury.ac.nz** or text/call **021 02692145**

Thank you for reading ☺

This project has been reviewed and approved by the University of Canterbury Human Ethics Committee

Appendix E: Figure 9 and 10 Copyright Permission

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December 10th, 2019

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Elisa Andersen
Editorial Assistant